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The prevalence of multiple sclerosis in the Hungarian city of Szeged

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Objectives – The aim of this study was to determine the prevalence of multiple sclerosis in a population in South Hungary. *Methods* – The diagnosis was established with the aid of the Poser diagnostic criteria and the degree of physical disability was determined on the Kurtzke expanded disability status scale (EDSS). The present medical state (EDSS score) was determined from outpatient clinical control tests. The prevalence, the average age at onset of the disease and the proportions of the various clinical forms were calculated, and the patients' disability status was estimated. *Results* – In 1996, the prevalence was 65/100,000, and the incidence from January 1, 1995 through December 31, 1996 was 7/100,000/year. *Discussion* – During a period of 2 years, the number of diagnosed patients has almost doubled. The disease can be recognized in an early stage with a minimal neurological deficit. The development of the diagnostics necessitates re-examinations with modern diagnostic procedures. During the last 3 years, the general practitioner system has been reorganized, and the working relationships between the clinic and family doctors have developed considerably. A comparison of the present findings with those in other countries with a similar climate revealed very similar prevalence data.

Key words: multiple sclerosis; prevalence; incidence

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The diagnostic criteria of multiple sclerosis have recently improved, and the prevalence of this disease can now be determined more precisely. Epidemiologists in many countries worldwide are reanalyzing the prevalence of multiple sclerosis (1–13). The better diagnostic methods permit an improved differentiation of neurological diseases and shed light on earlier stages of the disease. Multiple sclerosis with a later onset is easier to differentiate (by means of MRI imaging and up-to-date-CSF diagnostic methods) from vascular diseases that are more frequent at this age and may cause similar complaints. In 1996, a uniform nomenclature was introduced for the clinical course of this disease, and this necessitates a reclassification of the patients (14). In Hungary, Pálffy et al. (15) calculated the prevalence in Baranya County in 1983, which was 37/100,000. The prevalence in Fejér County in Hungary in 1992 was found by Guseo et al. (16) to be 69/100,000. Unfortunately, some of those patients did not undergo MRI examinations. The aim of the present study was to determine the prevalence in a relatively stable population with the Poser diagnostic criteria (17). The proportions of the various clinical forms were determined in accordance with the new nomenclature; such data have not been published from Hungary previously.

Methods

The Department of Neurology at Albert Szent-Györgyi Medical University in Szeged, Hungary, has a Multiple Sclerosis Outpatient Unit. The Unit is the only organization that deals with the medical care of multiple sclerosis in the city; the prevalence data can therefore be regarded as reliable. According to the files of the Hungarian Central Statistical Office (Központi Statisztikai Hivatal), the number of inhabitants in Szeged on January 7, 1997 was 198,682. The population in this area in the remaining time was fairly stable. The diagnoses were established with the diagnostic criteria of Poser et al. (17), and the degree of physical disability was determined on the Kurtzke (18) expanded disability status scale (EDSS), the most widely used scoring method (19, 20). The first examinations in Szeged were carried out between 1990 and 1994, partly retrospectively and partly prospectively.

From 1990 to 1993, the history of the multiple sclerosis patients (the age at onset, the clinical course of the disease, etc.) was taken from inpatient and outpatient medical records. The current medical state (EDSS score) was determined from the results of outpatient clinical control tests.

At the end of 1993, we had 77 patients with sclerosis multiplex in Szeged (21). By the end of 1996, the number of patients had almost doubled. We chose December 31, 1996 as the prevalence day. At that specific time the prevalence was 65/100,000. In 1995 and 1996, the incidence was determined, the patients were classified according to the nomenclature of the clinical course of the disease, and the prevalence was calculated. In 1995, 30 new patients were added to the records. Seventeen of these were first-attack patients and the other 13 were referred by general practitioners. Five patients moved from Szeged and are now cared for by other multiple sclerosis outpatient units. These 5 patients have therefore been lost from our study. None of the remaining patients died before the end of 1996. In 1996, 28 new patients were diagnosed, 12 of whom were first-attack patients, the remaining 16 being referred by general practitioners. The male:female ratio was determined, and the average age at onset was calculated. The condition of the patients was assessed on the basis of the EDSS score and they were recategorized according to the new clinical course nomenclature. The proportions of the clinical forms were determined.

Table 1. Criteria for the diagnosis of multiple sclerosis

	Number of attacks	Evidence of more than one lesion		CSF, OGP or IgG
		Clinical	Laboratory	
A. Clinically definitive				
A1	2	2		
A2	2	1 and	1	
B. Laboratory-supported definitive				
B1	2	1 or	1	+
B2	1	2		+
B3	1	1 and	1	+
C. Clinically probable				
C1	2	1		
C2	1	2		
C3	1	1 and	1	
D. Laboratory-supported probable				
D1	2	0	0	+

From Poser C, Paty DW, Scheinberg L, McDonald WI, Ebers GC. The Diagnosis of Multiple Sclerosis. New York: Thieme-Stratton, 1984.

Results

With the Poser diagnostic criteria (17, Table 1), the majority of the patients fell into the definitive category of multiple sclerosis, with a significant majority in the subgroup who were diagnosed both clinically and in the laboratory. The criteria for this subgroup are at least 2 lesions, proven clinically, positive MRI imaging, positive oligoclonal bands in the CSF with isoelectric focusing, and a positive Link index. Laser nephelometry analysis was used for the quantitative determination of proteins (Fig. 1). The calculations revealed a multiple sclerosis prevalence in Szeged of 65/100,000 inhabitants. The male:female ratio was 1:3. The average age at the onset of the disease was 35 years (Fig. 2).

As regards the clinical course, 80% of the patients had the relapsing–remitting form, 11% the primary chronic progressive form, 4% the benign form, 4% the secondary chronic progressive form and 1% the relapsing–progressive form (Fig. 3). In accordance with the EDSS scores the majority of the patients are able to live a normal life: 28 (21%) have no symptoms and 74 (57%) have only mild symptoms enabling them to perform everyday activities, 21 (16%) can carry out restricted activity, 2 (2%) are confined to a wheelchair and 5 (4%) are bed-ridden (Fig. 4). At the end of 1993, 77 of the approximately 200,000 inhabitants of Szeged suffered from multiple sclerosis, whereas in 1996 the prevalence was 65/100,000 (Table 2). The incidence from January 1, 1995 through December 31, 1996 was 7/100,000/year (Table 3).

All of the 17 new first-attack patients diagnosed in 1995, and all of the 12 new first-attack patients diagnosed in 1996, had the relapsing–remitting form. Six patients (3 males and 3 females) displayed a late age at onset, the first attack occurring after the age of 50.

Discussion

Dean (22) determined the prevalence of multiple sclerosis within the continental zone as 30–80/100,000. The occurrence was found to be related to geographical distribution, migration and genetic contribution (23–31). In research on the etiologic factors of multiple sclerosis, the occurrence and clinical forms of the disease have been examined in different population groups, such as mestizos, Indians, etc. (1, 29). A distinction between low, medium and high-risk factor areas can be made on the basis of geographical lines of latitude. However, the new prevalence tests reveal that in both low and medium-risk factor areas there can in fact be an enhanced occurrence (5, 6, 29). There are both pro and contra arguments for a north–south gradient distribution (11, 22). In 1961, Lehoczky and Halasi

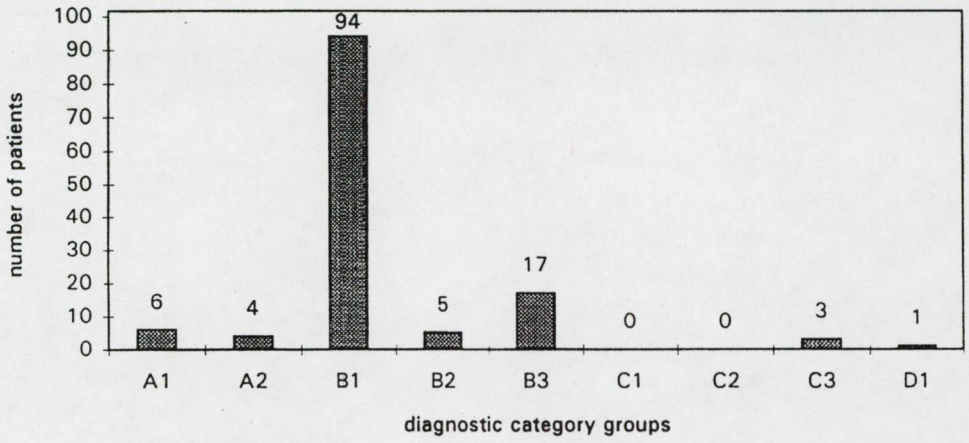

Fig. 1. Patients diagnosed with the Poser diagnostic criteria (total number of patients: 130)

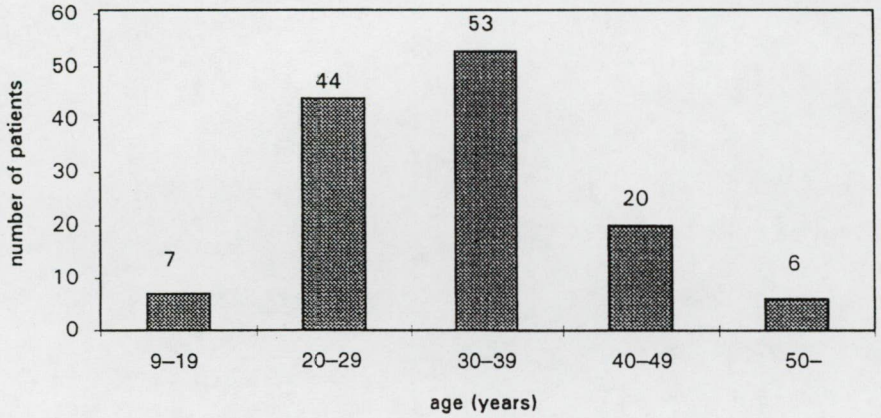

Fig. 2. Age at onset in multiple sclerosis patients (total number of patients: 130)

first determined the Hungarian prevalence of multiple sclerosis, which was found to be 20/100,000 (see in review by Pálffy et al. (15)). In 1983, Pálffy et al. (15) reported the prevalence in Baranya County as 37/100,000, and in 1992, Guseo et al. (16) found the prevalence in Fejér County to be 69/100,000. Hungary is a country lying on the Pannon plateau, situated in a continental temperate zone. Hungarians are of Asian ancestral descent, belonging in the Caucasian race. Through gene analysis, Hungarians with multiple sclerosis were found to be 32.3% HLA-B7-positive and 42.4% HLA-DRW2 histocompatibility-positive (15). In 1983, Pálffy described a male Gypsy with definitive multiple sclerosis and also examined his HLA structure (15). The Gypsy minority race, just like the Lapps of Scandinavia, the North American Indians of Canada, the Afro-Americans, and the Maoris of New Zealand, have been found to be resistant against multiple sclerosis (15). Gyódi et al. (30) compared the HLA types of the Gypsies in Baranya County with those of the Hungarian non-Gypsy population. There was a lack of HLA-B7 histocompatibility positivity in comparison with the Hungarian population, and also an increased frequency of HLA-DR2 antigen.

Our unit currently cares for 380 patients with multiple sclerosis, 130 of whom live in the city of Szeged. Since 1990, the occurrence has been calculated twice in this area; there has been a rise in the number of patients during this period, but no growth in incidence. The reason is that, even though all known multiple sclerosis patients in Szeged are treated by the unit, the patients examined prior to 1990 were not necessarily appropriately diagnosed because of the inadequacy of the diagnostic equipment. Re-examinations in the past 3 years led to an increased number of patients being taken into clinical care. A few patients were admitted to the clinic after several years of complaints that were inadequately diagnosed elsewhere. Our results relating to both prevalence and incidence do not differ significantly from the nationwide data.

The majority of the multiple sclerosis patients in Szeged can be classified into the definitive clinical and laboratory group, which means that the clinical

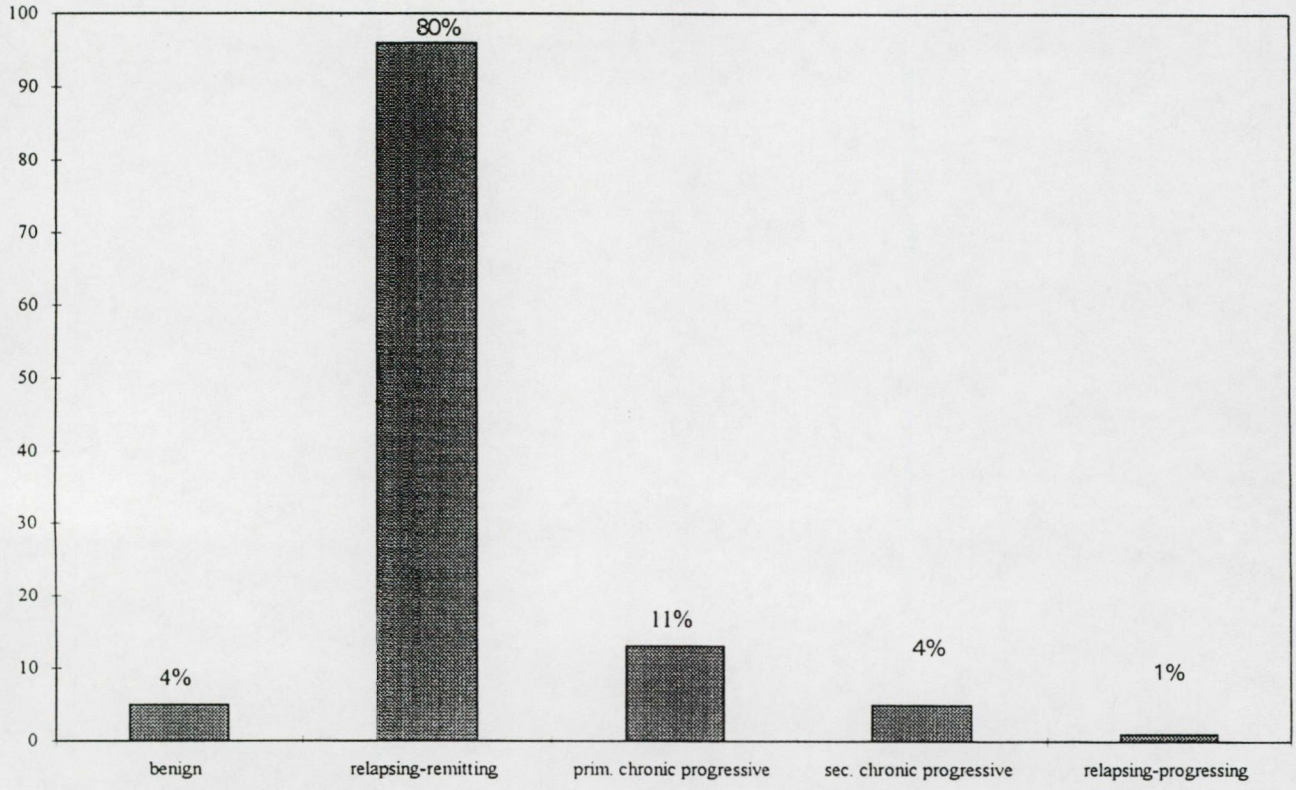

Fig. 3. Clinical forms of multiple sclerosis (total number of patients: 100%=130)

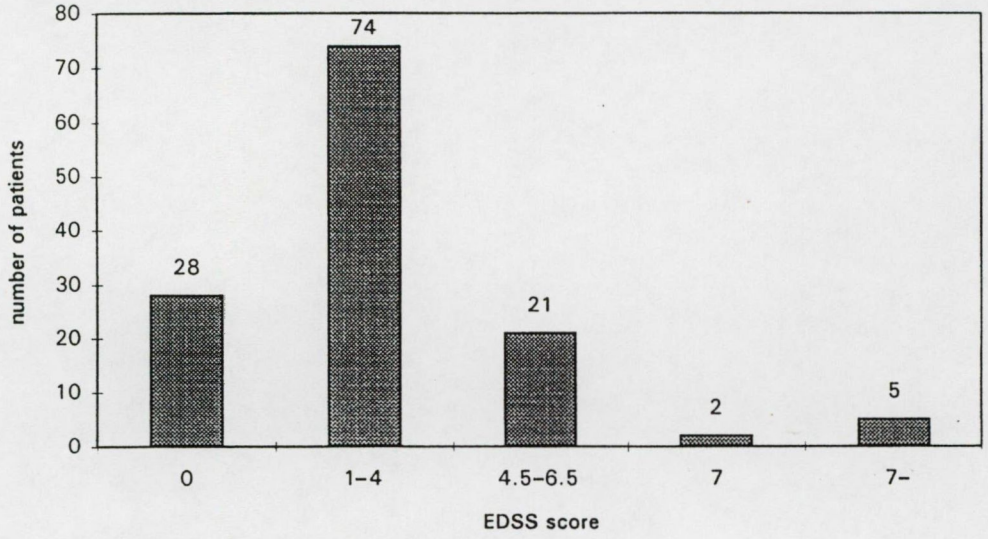

Fig. 4. EDSS scores in multiple sclerosis patients (total number of patients: 130)

outcome, the MRI results and the CSF data all indicate multiple sclerosis. Of the multiple sclerosis patients in Szeged, 21% are asymptomatic and a further 57% are capable of normal activities. This means that at present 78% of the patients are not yet impaired, 16% are not capable of full daily activity, 2% are confined to a wheelchair and 4% require help. Our population contains few 50–60-year-old multiple sclerosis patients, which suggests that some patients are presumably treated in non-neurological departments, or perhaps by family physicians, with other diagnoses.

Throughout the period of 2 years, the number of diagnosed cases has almost doubled. During the last 3 years, the general practitioner system has been reorganized, and the working relationships between

Table 2

	1996
Prevalence	65/100,000
Male:female ratio	1:3
	32 males, 98 females
Mean age at onset	35 years
	(range: 11–64 years)

Table 3

	1995–1996
Incidence	7/100,000/year male:female ratio 1:5 mean age at onset: 37 years (range: 18–64 years)

the clinic and family doctors have developed considerably. This fact, together with the young age of the patients, and their generally good functional condition led us to conclude that the diagnostic developments necessitated re-examinations with modern diagnostic procedures. Thus, the disease can be recognized in an early stage with a minimal neurological deficit. A comparison of our findings with those from countries with a similar climate revealed similar prevalence data.

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II.

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The Prevalence of Multiple Sclerosis, Distribution of Clinical Forms of the Disease and Functional Status of Patients in Csongrád County, Hungary

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Key Words
Multiple sclerosis · Prevalence · Incidence · Clinical forms · Functional status

Abstract
Objective: The aim of this study was to determine the prevalence of multiple sclerosis (MS) in the population of Csongrád County, Hungary (400,128 inhabitants) and to determine the functional status (based on the Expanded Disability Status Scale; EDSS) of the patients according to the clinical forms of the disease. ***Methods:*** The diagnosis was established with the aid of the Poser diagnostic criteria, and the degree of physical disability was determined using the Kurtzke EDSS. ***Results:*** In Csongrád County, the prevalence of MS is 62/100,000. The distribution of patients according to the clinical forms of MS was as follows: 15% had the benign form, 54% had relapsing-remitting MS, 20% had secondary chronic progressive MS and 11% had the primary chronic progressive form of MS. Sixty percent of relapsing-remitting MS patients had an EDSS score of 0–4 points and 33% had an EDSS score of 4.5–6.5 points. ***Conclusion:*** The distribution of patients according to the clinical forms of the disease in this representative population is comparable to results in other regions of the world.

Introduction

The aim of the present study was to determine the prevalence of multiple sclerosis (MS), the distribution of clinical forms of the disease and the Expanded Disability Status Scale (EDSS) scores [1] of MS patients in a large population to obtain exact data about the prevalence of the disease in Hungary. In order to provide new therapies for MS (beta-interferons, glatiramer acetate) the functional status (based on the EDSS score) of patients has to be determined in the different clinical forms. There are no published data on this topic in the population of 400,000 which we investigated in the present study, which comprises 4% of the residents of Hungary.

Pálffy et al. [2] calculated a prevalence of 37/100,000 in Baranya County, Hungary, in 1983, based on Bauer criteria.

In 1997, the prevalence of MS was 65/100,000 in the city of Szeged in Hungary, based on a population of 200,000 residents [3]. In this population, there was a relatively low proportion of patients with the secondary chronic progressive (4%) and benign forms (5%) of MS compared to the international data [4, 5].

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Subjects and Methods

The current study was carried out in the Department of Neurology at Szeged University, Hungary (January 1, 1997 to July 1, 1999). The Multiple Sclerosis Outpatient Unit is the only facility that has dealt with the medical care of MS in the city for the past 35 years. This outpatient unit of the Neurological Department as a university center is specialized in caring for and nursing the MS patients of the south Hungarian region, which comprises 4 counties. With the background of the university, it has a wide range of tools to diagnose MS patients.

In our first epidemiological study in the city of Szeged [3], we checked the medical records of all general practitioners, neurological and ophthalmological departments and homes for the aged in the city. Based on these medical records (onset of disease and the clinical course), patients with probable MS were registered and examined by our outpatient unit. The diagnoses were established using the diagnostic criteria of Poser et al. [6]. Brain MRI was performed in all patients, and a proportion of the patients had optic nerve and/or spinal cord MRI and analysis of CSF. Oligoclonal bands were determined by isoelectric focusing, and laser nephelometry analysis was used for the quantitative determination of proteins to calculate the Link index. In all cases of CSF analysis, we had the informed consent of the patients. The criteria for A subgroups are 2 attacks and 2 lesions proven clinically (A1) or 1 clinical and 1 paraclinical sign of lesion (A2). The criteria for B subgroups are at least 1 lesion (B1) or 2 lesions (B2) proven clinically and positive MRI imaging, positive oligoclonal bands in the CSF and a positive Link index [7]. The degree of physical disability was determined using the Kurtzke [1] EDSS.

The outpatient unit has had an MS register since 1996, with up-to-date patient records of the Szeged patient population. We examine patients every 3 months to determine their neurological status and EDSS score. In the case of relapses, we perform an extra neurological examination and, if necessary, admit the patients to hospital. After discharge, a follow-up visit is scheduled for 2–4 weeks later, at which we check the neurological status and the EDSS score.

If a patient moved away from the city or died, we updated the register. At the end of the year, we calculate the yearly incidence of the disease.

Based on the methods used in this register, we performed a wider survey in Csongrád County as a whole.

According to the files of the Hungarian Central Statistical Office, Szeged City (the county town of Csongrád County) has 198,686 inhabitants, and Csongrád County has an additional 222,506 inhabitants. In the present study, 400,128 inhabitants were the representative sample; the population of a small city with 21,064 residents was excluded because there were no available data for these persons.

We used the same method described above to record and diagnose MS patients in the case of 201,442 inhabitants of Csongrád County.

We chose July 1, 1999 as the day on which we measured prevalence. We determined the prevalence of the disease and the male/female ratio. We estimated the following parameters for the different clinical forms of the disease: distribution of patients; average age at the onset of the disease; average duration of the disease; average age on the prevalence day, and the EDSS score based on the most recent examination (April 1, 1999 to July 1, 1999).

Results

We found 248 MS patients alive on the prevalence day in Csongrád County. Two hundred and eighteen patients had clinically definite MS. According to the criteria of Poser et al. [6], 208 patients were in the A1 and 10 patients in the A2 (with negative CSF oligoclonal bands and Link index) category. Thirty patients were in the category of laboratory-supported definite MS (Poser B2). Although it is not essential for the A1 category, our patients belonging to this subgroup had positive CSF findings, that is, oligoclonal bands and a positive Link index. Based on our study, the prevalence of MS in Csongrád County is 62/100,000. On the prevalence day, 130 patients out of 248 lived in Szeged. Based on our MS register, we diagnosed 26 new MS patients in the city of Szeged between January 1, 1997 and July 1, 1999. In this period, 5 patients died, 12 patients moved from Szeged to elsewhere within the county and 9 moved away from the county. The incidence of MS in the city of Szeged was 5/100,000 in 1997 and 6/100,000 in 1998. Up to July 1, 1999, we diagnosed 4 new cases.

There were 66 (27%) male and 182 (73%) female MS patients found, giving a male/female ratio of 1:2.75.

The distribution of the patients according to the clinical forms of MS was as follows: 15% had the benign form, 54% had relapsing-remitting MS, 20% had secondary chronic progressive MS and 11% had the primary chronic progressive form of MS (table 1).

The EDSS score in patients with the benign form of MS was 0–3 points; the average duration of the disease was 27 years. Sixty percent of relapsing-remitting MS patients had an EDSS score of 0–4 points and 33% had an EDSS score of 4.5–6.5 points. Fifty-six percent of secondary chronic progressive MS patients had an EDSS score of 4–6.5 points; 44% were confined to a wheelchair or bedridden (EDSS score ≥ 7) (table 2).

Discussion

Dean [8] determined the prevalence of MS within continental Europe as 30–80/100,000. The occurrence of MS has been found to be related to geographical distribution, migration and genetic contribution [6, 9–12]. A distinction between low-, medium- and high-risk factor areas can be made on the basis of geographical lines of latitude. However, the new prevalence tests reveal that in both low- and medium-risk factor areas, there can be, in fact, a higher rate [13–20]. There are both pro and con arguments for

Table 1. Patient characteristics according to clinical forms of MS

	Benign MS	Relapsing-remitting MS	Secondary chronic progressive MS	Primary chronic progressive MS
Number of patients	37 (15%)	137 (54%)	48 (20%)	26 (11%)
Mean age at the onset of disease, years	28 (16–41)	28 (16–40)	30 (13–47)	52 (42–62)
Mean age on the prevalence day, years	55 (38–72)	36 (18–54)	59 (39–69)	59 (49–68)
Average duration of disease, years	27 (10–34)	8 (0–15)	29 (5–47)	7 (2–13)

Figures in parentheses represent ranges, except where otherwise indicated.

Table 2. Distribution of patients according to clinical forms of MS and EDSS score

Benign MS			Relapsing-remitting MS			Secondary chronic progressive MS			Primary chronic progressive MS		
EDSS	n	%	EDSS	n	%	EDSS	n	%	EDSS	n	%
0	10	27	0–4	82	60	4–6.5	27	56	3.5	1	4
1	5	13	4.5–6.5	45	33	≥7	21	44	4	2	8
1.5	1	4	≥7	10	7				4.5–6.5	12	46
2	11	30							≥7	11	42
2.5	5	13									
3	5	13									

a north-south gradient distribution [21]. Rothwell and Charlton [22] found a high prevalence rate for MS in southeast Scotland, suggesting that the Scottish population as a whole has a genetic susceptibility to the disease.

In 1983, Pálffy et al. [2] reported the prevalence of MS in Baranya County, Hungary, as 37/100,000. In 1997, the prevalence in Szeged City was found to be 65/100.000 [3]; in 1999, the prevalence was 62/100,000 in Csongrád County, including the population of Szeged City. The prevalence of MS found in the 400,128 persons of Csongrád County in the present study is nearly the same as the previous prevalence data from Szeged City and the prevalence of MS in other communities of the same geographical regions.

The male/female ratio of 1:3 found in Szeged City differs from the international data [4, 5], because in that city, the ratio of females is higher by 8.5% than males. The mean age is lower by 5.5 years in the city than the rest of the county. In Csongrád County, the male/female ratio is 1:2.75, which is comparable to the international data.

In Szeged City, the distribution of patients according to the clinical forms of the disease was as follows: 5% had the benign form, 80% had relapsing-remitting MS, 4% had secondary chronic progressive MS and 11% had the primary chronic progressive form of MS.

In Csongrád County, we found that 15% of patients had the benign form of MS, 54% had relapsing-remitting MS, 20% had secondary chronic progressive MS and 11% had the primary chronic progressive form.

The proportion of secondary chronic progressive MS patients in the county as a whole is higher (20%) than in the city of Szeged (4%). Most of these MS patients were found in homes for the aged in the county. Due to progression of the disease and worsening social status, these chronic progressive MS patients moved to smaller communities away from the city. Therefore, the population of the city is younger than in the rest of the county (difference in average age is 5.5 years). In the current and previous epidemiological study [3] of this region, the proportion of patients with primary chronic progressive MS was 11%, which is equal to international data.

The average duration of the disease in the benign form of MS was 27 years, and the EDSS score for these patients was between 0 and 3. These data fit the criteria of benign MS.

Sixty percent of relapsing-remitting MS patients in this study were capable of normal activities; only 7% were confined to a wheelchair and required help. Of the relapsing-remitting MS patients, 21% were being treated with interferon-β-1b or glatiramer acetate, thus modifying the EDSS score.

Of the secondary chronic progressive MS patients, 56% had an EDSS score between 4 and 6.5; 44% were confined to a wheelchair and required help.

In the larger population of Csongrád County (more representative population than Szeged City), the distribution of patients according to the clinical forms of the disease was comparable to international results [23].

In order to adopt and finance the new therapeutic approaches (interferons or glatiramer acetate), the Central-Eastern European healthcare systems need the most exact data possible on the prevalence of MS. The EDSS classification of the disease by clinical forms is necessary to estimate healthcare expenditures for these new, expensive therapies.

The sample investigated in the current epidemiological study represents 4% of the Hungarian population, which could be quite sufficient to estimate national expenditure.

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Catecholamine levels in peripheral blood lymphocytes from multiple sclerosis patients

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Abstract

Circumstantial evidence suggests the involvement of sympathoadrenergic mechanisms in the progress of multiple sclerosis (MS). We studied peripheral blood lymphocytes from MS patients. The levels of dopamine (DA), norepinephrine (NE), epinephrine (E) and their metabolites in extracts of lymphocytes from 58 MS patients and 19 healthy controls were measured by using capillary electrophoresis. The MS patients were divided into clinical subgroups: a laboratory-supported definitive (first-attack) MS group, and a relapsing–remitting (RR) group in remission. The peripheral blood lymphocyte level of epinephrine was significantly higher in the first-attack MS patients ($p=0.028$) than in the controls. However, the norepinephrine levels were significantly ($p=0.027$) lower in the RR patients in remission. The catecholamines are known to be able to affect the lymphocyte activity, both by stimulation and by immunosuppression. Our results suggest that the catecholamines are important regulators of lymphocyte activation in MS, and of potential importance as concerns new diagnostic and therapeutic methods.

Keywords: Capillary electrophoresis; Catecholamine; Dopamine; Epinephrine; Lymphocytes; Multiple sclerosis; Norepinephrine; Peripheral blood mononuclear cells

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS). Current hypotheses on the pathogenesis of MS suggest that the primary peripheral activation of autoreactive T helper-1 lymphocytes precedes the recognition of CNS auto-antigens. These T cells proliferate, secrete cytokines and cross the blood–brain barrier (BBB) to find their antigens in the CNS where they cause further inflammatory damage. It has been hypothesized that relapsing–remitting (RR) MS is driven by a systemic antigen presentation and that chronic progressive MS depends on the CNS presentation of antigens (Hafler, 1999).

Studies involving experimental models of MS demonstrate the importance of lymphocytes and sympathoadrenergic mechanisms (Anderton et al., 1999). Thus, in experimental autoimmune encephalomyelitis (EAE) lymphocytes crossing the BBB undergo a transformation that is involved in the progress of the disease (Wekerle, 1993). Experimentally induced hypercatecholaminemia in rats seems to protect the lymphocytes from the immunosuppressing effects of other endogenous stress hormones, but causes suppression of peripheral blood lymphocyte activation if the β-receptors are blocked at the same time. Beta-adrenergic agonists suppress chronic/relapsing EAE (Wiegmann et al., 1995) and decrease the number of β-adrenergic receptors on splenic lymphocytes in Lewis rats (Muthyala et al., 1995).

As an immune privileged site, the brain is not totally separated from the immune system, as thought earlier. The

Abbreviations: BBB, blood brain barrier; CNS, central nervous system; CSF, cerebrospinal fluid; DA, dopamine; E, epinephrine; MS, multiple sclerosis; NE, norepinephrine; PBMC, peripheral blood mononuclear cell; RR, relapsing-remitting.

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CNS is connected to the deep cervical lymphatic nodes and shares messengers with the immune system. One group of these common transmitters is the catecholamines. Immunocompetent cells have been shown to contain and produce catecholamines, serotonin (5-HT), melatonin and acetylcholine (Bergquist et al., 1994; Josefsson et al., 1996; Dedenkov et al., 1986; Musso et al., 1996; Rinner et al., 1998), together with many neuropeptides and hormones, and also to express their corresponding receptors (Blalock, 1992; Santambrogio et al., 1993; Felten et al., 1992; Ricci et al., 1995; Felsner et al., 1995; Costa et al., 1995). Catecholamines and their metabolites have been found in the lymphocytes in a number of studies (Bergquist et al., 1994, 1997; Josefsson et al., 1996; Musso et al., 1996, 1998; Bergquist and Silberring, 1998); the amount of intracellular dopamine (DA) is approximately 10^{-18} mol/cell (Bergquist et al., 1994).

Lymphocytes have a cellular uptake mechanism, but are also capable of the endogenous synthesis of DA and norepinephrine (NE). Additionally, they are also able to store and degrade catecholamines and possibly to regulate their own activity via an autocrine loop. Furthermore, catecholamines have been found inside the nuclear envelope (Bergquist et al., 1998), suggesting a possible direct interaction with the transcription machinery or via an interaction with the nuclear factor-κB (NF-κB) regulatory system (Bergquist et al., 2000). Recent results suggest a crucial role of NF-κB1 in the activation and differentiation of autoreactive T cells. Blocking the NF-κB function can be an effective way to prevent autoimmune encephalomyelitis (Hilliard et al., 1999). Elevated regional levels of catecholamines might lead to suppression and finally apoptosis, which would partly explain the immune privilege of the brain (Bergquist et al., 1994, 1997, 1998).

The catecholamines secreted by the sympathetic nervous system predominantly act on human T cells of the $CD8^+$, $CD28^-$ (suppressor) subset (Karaszewski et al., 1991). This subset has the highest β-adrenergic receptor density. NE stimulates, while norepinephric denervation diminishes the Th1 responses (cellular immunity). Humoral immunity is also affected, perhaps via additional signaling to B cells, NE favoring IgM responses and noradrenergic denervation favoring a shift from IgM to IgG responses (Felten and Felten, 1994).

The discovery of catecholamines in lymphocytes and their functional role involving the control of T and B lymphocytes (Bergquist et al., 1994) led to many questions being raised about their role in neuroimmunological interactions. The regulation of lymphocyte functions by catecholamines could prove to be an important part of immune deactivation in the nervous system. Studies on human neutrophils and peripheral blood mononuclear cells (PBMCs) demonstrated a catecholamine lifecycle in these cells, suggesting the presence of autoregulatory adrenergic mechanisms (Bergquist et al., 1998; Cosentino et al., 1999; Marino et al., 1999).

In the present study it was hypothesized that the deactivation of the immune system after a MS relapse (remission) could be mediated by catecholamines. Accordingly, the intracellular levels of catecholamines in relapsing–remitting (RR) MS patients in remission and in first-attack MS patients are described.

2. Subjects and methods

2.1. Patients and controls

A total of 58 patients were examined and were found to have clinically and laboratory-supported definitive MS according to the Poser criteria (Poser et al., 1983); 10 were laboratory-supported definitive (first-attack) patients, and 48 had RR MS. Both the cerebrospinal fluid (CSF) findings (oligoclonal bands on isoelectric focusing electrophoresis) and the MRI findings (several periventricular T2-weighted lesions) of the first-attack patients supported the MS diagnosis. All the RR patients were in remission. None of the patients had received steroid therapy within 30 days and none of them were on tricyclic antidepressants, cardiac drugs or amantadine. The neurological conditions of the patients were expressed on the Kurtzke expanded disability status scale (EDSS) (Kurtzke, 1983). Healthy individuals ($n=19$) served as controls. The study was approved by the ethical committee of Albert Szent-Györgyi Medical School at the University of Szeged (886/1998). For the statistical analysis various MS subgroups were formed, depending on (a) the clinical course of the disease: first-attack (10) or relapsing–remitting (48); (b) the EDSS score: EDSS score <4.0 (49) or >4.0 (9); (c) the duration of the disease: <5 years (30) or >5 years (28); (d) the time to the last relapse: relapse period <6 months (19) or >6 months (39). More data on the patients are provided in Table 1.

Table 1
Patient's data

Group	No. of subjects	Last relapse (months)	No. of relapses/2 years	Onset (year)	EDSS	Age (year)
First-attack MS	10	14.6 ± 3.0	0.9 ± 0.1	0	0.0	38 ± 2
RR MS	48	16.8 ± 1.8	1.0 ± 0.14	2.5 ± 0.07	2.2 ± 0.05	40 ± 1
Relapse within 6 months	19	3.9 ± 0.4	1.5 ± 0.21	2.4 ± 0.11	2.2 ± 0.08	40 ± 2
Relapse-free for >6 months	39	22.5 ± 0.8	0.8 ± 0.12	2.5 ± 0.08	2.1 ± 0.06	40 ± 1
Controls	19	–	–	–	–	30 ± 2

2.2. Preparation of lymphocytes

Peripheral vein blood samples (12 ml) were prepared by centrifugation at 2500 × *g* for 10 min. Lymphocytes were isolated by centrifugation on a Lymphoprep® (Nycomed Pharma, Oslo, Norway) density gradient and, after washing and centrifugation steps, kept at − 80 °C until analysis. The lymphocytes were extracted by adding 25 μl perchloric acid (containing 1 mM NaEDTA and 1 mM Na_2SO_3) to the pellet, followed by ultrasonication on ice for 2 min using a MSE Soniprep 150 probe. After centrifugation (30 min, 4 °C, 35 000 × *g*) the supernatant was frozen and stored at − 80 °C until analysis. The pellet was used for spectrophotometric protein quantitation, using bicinchoninic acid protein assay reagent (BCA, Pierce Chemical, Rockford, USA).

2.3. Capillary electrophoresis with electrochemical detection

The capillary electrophoretic system used was described in detail earlier (Bergquist et al., 1994, 1998; Josefsson et al., 1996). Briefly, a buffer-filled fused silica capillary (Polymicro Technologies, Phoenix, USA) measuring 10 μm in i.d. and 65 cm in length was placed between two buffer reservoirs. High voltage was applied at the injection end, and the reservoir containing the detector end was held at ground potential. Electrokinetic injection was used for all sample introductions, 5 s at 30 kV; the sample volume was approximately 600 pl. The easily oxidized analytes were detected in the amperometric mode with a two-electrode configuration, using optimized end-column detection (Bergquist et al., 1997). A carbon-fiber microelectrode was inserted into the end of the electrophoresis capillary and held at 0.8 V versus a sodium-saturated calomel electrode. Reagents: 2-(*N*-morpholino)ethanesulfonic acid (MES), 5-HT, NE, epinephrine (E), DA, L-dihydroxylphenylalanine (L-DOPA), vanilmandelic acid (VMA), methoxyhydroxyphenyl glycol (MHPG), homovanilic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) were obtained from Sigma (St. Louis, USA) and used in the form received. The electrophoresis buffer was 25 mM MES adjusted to pH 5.65 with NaOH. Calibration standards were prepared as 10 mM stock solutions in perchloric acid and diluted to the desired concentration in electrophoresis buffer. Hydrofluoric acid was obtained as a 40% aqueous solution from Aldrich, Milwaukee, USA), and was used for the etching of the detector end of the capillary.

The catecholamine levels of the lymphocytes were quantified by direct comparison with the standard electropherograms run before and after the patients' samples. The catecholamine contents of the lymphocytes are given in fmol/μg protein. Detection limits were determined (for DA, NE 0.13 fmol/μg protein, for E 0.37 fmol/μg protein, and for DOPAC 0.11 fmol/μg protein) and estimated at twice the peak-to-peak noise level by extrapolation from plots of peak area versus concentration. Between the series of runs, the capillary was flushed with 0.1 M NaOH to refresh the inner capillary surface and to maintain reproducible separation conditions. For a more detailed description of the method, see Bergquist et al. (1994).

2.4. Statistical analysis

The Kruskall–Wallis test (SPSS 7.5 for Windows) was performed for statistical analysis to compare the catecholamine levels in the healthy controls and the subgroups of

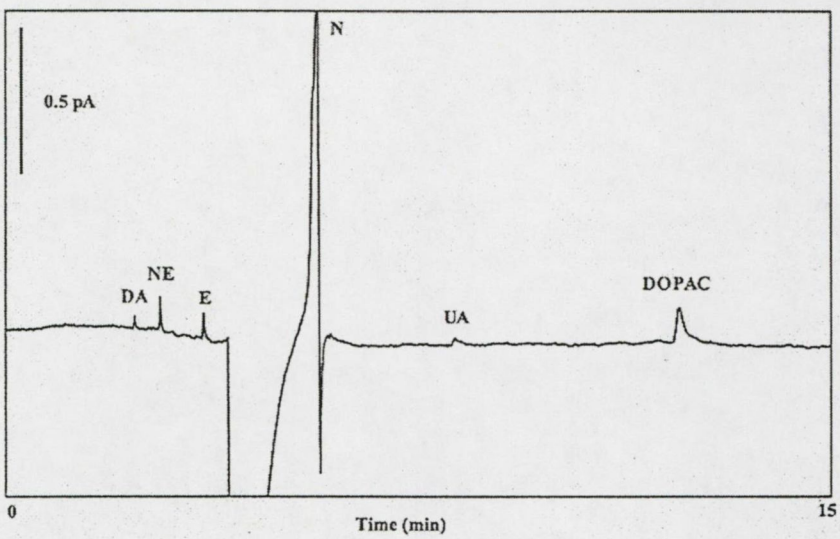

Fig. 1. A representative electropherogram of catecholamines extracted from human peripheral blood lymphocytes, showing the peaks of dopamine (DA), norepinephrine (NE), epinephrine (E), neutral species (N), uric acid (UA) and dihydroxyphenylacetic acid (DOPAC).

MS patients, followed by the Mann–Whitney *U*-test for pairwise comparisons to asses the differences between the patients and the healthy controls. The Kruskal–Wallis test was also used for the statistical analysis of the differences between the healthy controls and the different MS subgroups regarding EDSS score, medication, and duration.

3. Results

The electrophoretic mobilities of the major peaks in the electropherogram corresponded to the calculated electrophoretic mobilities of DA, NE, E, uric acid (UA), and DOPAC (Fig. 1). We excluded the 5-HT, MHPG, VMA and ascorbic acid data because their levels were often under the detection limit (MHPG was detectable in 7/19 controls and in 19/58 MS patients, and VMA in 5/19 controls and 27/58 MS patients). We also excluded L-DOPA since it is a neutral molecule and has the same electrophoretic mobility as all other neutrals, therefore leading to difficulties with the quantification. The levels of the catecholamines are presented in Table 2.

3.1. Healthy controls versus first-attack and RR MS patients

When the MS patient subgroups and the healthy individuals were compared, significantly lower levels of NE (Kruskal–Wallis test, $p=0.027$) and higher levels of E (Kruskal–Wallis test, $p=0.028$) were found in the lymphocytes. Pairwise comparisons with the Mann–Whitney *U*-test showed that the RR MS patients ($p=0.017$) and the first-attack MS patients ($p=0.035$) had lower levels of intracellular NE than healthy controls (Table 2). The E content of the lymphocytes in first-attack MS patients was higher as compared to either the RR MS group ($p=0.008$) or the controls ($p=0.056$).

3.2. Differences between healthy controls and MS subgroups regarding EDSS scores, duration of disease and medication

Both the MS patients with shorter disease duration ($n=30$, mean ± SEM: 378 ± 90 fmol/μg) and those with longer disease duration ($n=28$, mean ± SEM: 453 ± 154 fmol/μg) displayed lower intracellular NE levels (Mann–Whitney *U*-test, $p=0.033$) as compared with the control group ($n=19$, mean ± SEM: 1594 ± 599 fmol/μg). The lymphocytes of both the patients in a better neurological condition ($n=49$, mean ± SEM: 368 ± 64 fmol/μg) and those with an EDSS score >4 ($n=9$, mean ± SEM: 807 ± 516 fmol/μg) contained less NE ($p=0.036$) than the cells of the controls ($n=19$, mean ± SEM: 1594 ± 599 fmol/μg). The administration of anxiolytics did not exert any significant effect on the catecholamine levels of the lymphocytes. Slight, nonsignificant differences in the NE contents of the lymphocytes were found between the group without immunomodulating medication ($n=42$, mean ± SEM: 332 ± 56 fmol/μg), those receiving interferon-β-1b treatment ($n=9$, mean ± SEM: 450 ± 235 fmol/μg), those receiving glatiramer acetate treatment ($n=7$, mean ± SEM: 1039 ± 649 fmol/μg) and the controls ($n=19$, mean ± SEM: 1594 ± 599 fmol/μg).

4. Discussion

Modern analytical tools such as capillary electrophoresis techniques allow the detection of intracellular catecholamine levels and give an insight into their regulation of lymphocyte differentiation, proliferation and apoptosis. The increased beta-adrenergic receptor density on the lymphocytes of MS patients in relapse suggests an involvement of lymphocytes and catecholamines in the pathogenesis of the disease. A general problem in MS research is that the phenomena observed can either be secondary to the disease progress with no causality, or reflect mechanisms of importance for the disease.

Scattered reports suggest a role for low molecular weight neurotransmitters in the pathogenesis of MS. Elevation of the levels of NE by using antidepressants and L-DOPA has been found to affect the symptoms of MS (Berne-Fromell et al., 1987). Maprotilin and lofepramin enhance the levels of NE in the synapses (Baumann and Maitre, 1979). Seventy-five percent of MS patients treated with L-DOPA experienced an improvement after 1–2 months (Berne-Fromell et al., 1987). Numerous

Table 2
Catecholamine contents of peripheral blood lymphocytes in healthy individuals and various subgroups of MS patients

Test group	No. of subjects	DA	NE		E		DOPAC
Healthy individuals	19	1.39 ± 0.32	1.59 ± 0.60	*50.0	0.06 ± 0.02	*43.8	2.02 ± 1.41
RR (first-attack MS)	10	1.89 ± 0.85	0.24 ± 0.10[a]	*30.0[b]	0.18 ± 0.08[c]	*62.9[d]	2.14 ± 2.08
RR (MS in remission)	48	1.68 ± 0.33	0.48 ± 0.11[a]	*36.0[c]	0.23 ± 0.14[c]	*40.0	6.29 ± 2.71

Values given as mean ± SEM fmol/μg protein and as *mean ranks.
RR = relapsing–remitting, DA = dopamine, NE = norepinephrine, E = epinephrine; DOPAC = dihydroxyphenylacetic acid.
[a] Significant difference between the first-attack and RR MS patients and healthy controls with Kruskal–Wallis test, $p=0.027$.
[b] Significant difference between first-attack MS patients and healthy controls with Mann–Whitney *U*-test, $p=0.035$.
[c] Significant difference between the first-attack and RR MS patients and healthy controls with Kruskal–Wallis test, $p=0.028$.
[d] Significant difference between first-attack and RR MS patients with Mann–Whitney *U*-test, $p=0.008$.
[e] Significant difference between RR MS patients and healthy controls with Mann–Whitney *U*-test, $p=0.017$.

studies have revealed that NE may regulate early immune events such as antigen localization, presentation, B cell activation, inhibition of T suppressor cell activation and the functions of both Th1 and Th2 cell function (Sanders, 1998; Madden and Livnat, 1991). NE may also suppress the normal immune response (Bergquist et al., 1998). Elevated levels of NE have been observed in the CSF, but not in the blood of MS patients (Barkhatova et al., 1997). It has been hypothesized that there is a deficiency of NE in the nerve terminals in MS, similar to the DA deficiency in Parkinson disease patients. This hypothesis is supported by the fact that near the fourth ventricle lies the locus ceruleus, a NE-mediated part of the brain regarded as a "stress center". Lower levels of NE in MS could possibly explain the reduced awareness and memory function, the difficulties with micturition and the cerebellar symptoms, which are the opposite of the "fight or flight" reactions (Berne-Fromell et al., 1987). Recent MRI and neuropathological findings suggest early axonal damage in MS that could be prognostic for the further disease progression (Ferguson et al., 1997; Trapp et al., 1998; Raine et al., 1999; Lovas et al., 2000). If the neurons are damaged, there could be an ungoverned release of catecholamines and high local concentrations in the area of the lesion. The lymphocytes in the region may be exposed to these high concentrations causing high intracellular levels by an initial uptake (as may be possible in first-attack).

We found changed intracellular catecholamine levels in the PBMCs of the MS patients. The analyzed changes reflect the whole PBMC population and probably only a small proportion of them are directly involved in the CNS pathogenesis. However, if the effect of immune regulation in MS is more systemic, this could be measured in the periphery. Normally just a few leukocytes are present in the CSF and the collection of these cells would be very difficult and demand single cell analysis. After considering these problems, we concentrated on collecting PBMCs. The inclusion criteria for first-attack patients were several T2-weighted lesions on the brain MRI and positive CSF findings (oligoclonal bands, elevated IgG levels in the CSF, and a positive IgG immune blot). In the city of Szeged, the incidence of MS in 1996 was 7/100.000 (Bencsik et al., 1998). Because of the low number of first-attack MS patients, it was difficult to add more data to this group.

Catecholamines also affect the natural killer cell function through β-adrenergic receptors (Takamoto et al., 1991). Activated lymphocytes have increased numbers of muscarinic and nicotinergic receptors (Besedovsky and DelRey, 1996). A number of reports suggest involvement of the catecholaminergic system in MS. A two-fold increase in β-receptor density was found on the PBMCs during relapse in RR MS patients and in secondary chronic progression MS, while the levels of NE and E in the plasma were similar to the control levels (Zoukos et al., 1992). From patients with

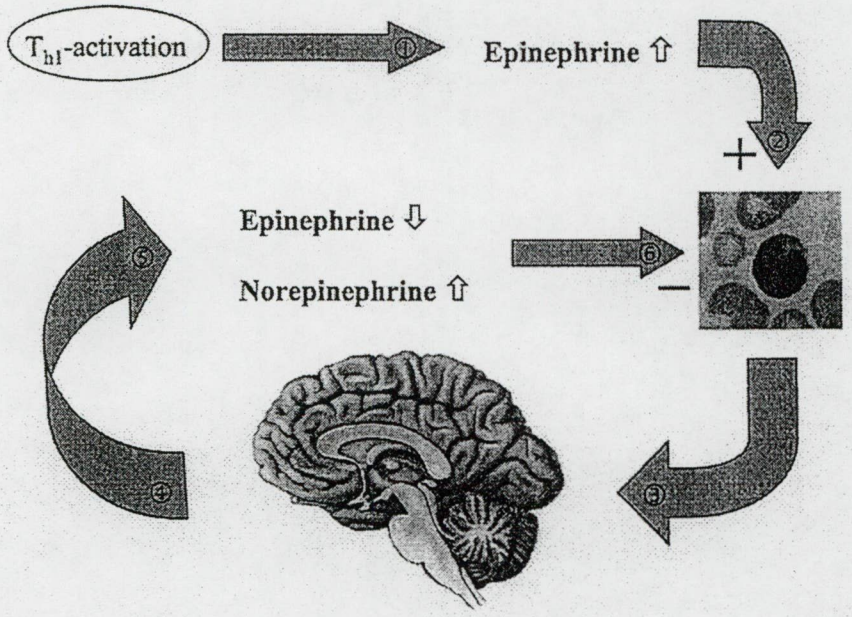

Fig. 2. The hypothesized role of the catecholamines in the pathogenesis of MS.① Th1 T cell activation takes place in the periphery.② Increased epinephrine levels activating the lymphocytes augment the entrance of lymphocytes through the BBB.③ Well inside the CNS, the lymphocytes find their antigens and are activated.④ After the activation of the lymphocytes, a feedback process is initiated.⑤ This feedback loop leads to lymphocyte deactivation and the epinephrine content of the lymphocytes is decreased. This epinephrine decrease is then followed by an increase in norepinephrine, causing a down-regulation of the lymphocytes ⑥, and leading to the steady state of remission.

chronic progressive MS, an increased number of β-adrenergic receptors was found on the CD8+ T cells. In contrast, patients with stable MS and those with relapsing–remitting disease before, during or after attacks had unchanged receptor densities (Karaszewski et al., 1991). The plasma E levels in samples drawn from patients in supine and upright positions were similar in chronic progressive MS to those for normal individuals, but the supine plasma NE levels were higher in chronic progressive MS (Karaszewski et al., 1993).

In a recent study, the percentages of T and B cells in the peripheral blood from MS patients in relapse, with viral inflammatory or with noninflammatory neurological disease were similar (Oreja-Guevara et al., 1998). Various cell surface molecules on the peripheral blood CD4+ T cells and the disease activity (by MRI examination) were monitored in relapse and in remission, but no differences and no correlation to disease activity could be found (Stuber et al., 1996).

No differences in plasma dopamine-β-hydroxylase activity have been reported between healthy individuals and MS patients (either in relapse or in remission) (Markianos et al., 1991). The synthesis of catecholamines in lymphocytes is under nicotinic control and acetylcholine might regulate catecholamine synthesis through activation of the rate-limiting enzyme tyrosine hydroxylase (Musso et al., 1997). We have not encountered any other data on differences in enzyme activity related to the catecholamine metabolism in the lymphocytes or peripheral blood of MS patients.

We observed higher intracellular levels of epinephrine in first-attack MS patients, and the lymphocytes express primarily β-adrenergic receptors. Thus, we can propose the following hypothesis, presented schematically in Fig. 2. An increased level of E activates the lymphocytes; they cross the BBB and find their antigens. This process is followed by the production of cytokines, which either result in an inflammatory process or act as the major compartment in the relapse process. A relapse-increased β-receptor density on the lymphocytes has been described, lending support to our hypothesis (Zoukos et al., 1992). It is not clear whether the lymphocytes merely mirror the state of the disease, reflecting the altered hypothalamus–pituitary gland–adrenal medulla (HPA) axis function and drain the catecholamines from the plasma, or are active participants, eliminating the catecholamines by uptake and degradation or releasing them into the MS plaque. The lower level of NE in the peripheral blood lymphocytes of RR MS patients in remission could be due to the β-adrenergic receptor down-regulation after a bout or to the degradation of the catecholamines. Remission may be due to a general down-regulation of the immune response by immunologically nonspecific mechanisms, such as the endogenous secretion of corticosteroids. Later in the disease process, a negative feedback suppresses the production of the catecholamines, resulting in a decreased catecholamine content of the peripheral blood lymphocytes during remission. This may explain why RR MS patients in remission may have lower levels of catecholamines such as NE and also account for the neuroimmunological entity of the relapse.

Higher catecholamine levels in the peripheral blood lymphocytes might prevent relapses. Catecholamines have a relatively short duration of action, which could be triggered by widespread activation, except when the levels are chronically changed. One of the risk factors for autoimmunity is the low NE level in MS patients, which reflects the hypoactivity of the HPA axis.

Relapses can be induced by infection, stress, or an elevated level of E, which activates the lymphocytes, resulting in turn to activation of the disease. After nicotinic activation of the lymphocytes, intracellular NE and L-DOPA production occurs (Musso et al., 1997). The catecholamine levels may play an important regulatory role, especially in RR MS patients, when the β-receptors on the lymphocytes are increased. This needs to be further investigated before any strong conclusions may be drawn.

MS patients have a significantly lower NE content in their peripheral blood lymphocytes than that for healthy individuals, but in the early stage of the disease, and hence in first-attack patients, the E content is higher. With regard to the fact that the lymphocytes in relapse have a higher β-receptor density, new means of early intervention in the pathogenesis of MS at the lymphocyte level may be possible. These data suggest a connection between the peripheral blood lymphocyte catecholamine content and the course of the disease, and may contribute to a better understanding of the pathogenesis of MS. They may also suggest a new therapeutic approach through recognition of the role played by lymphocytes in this disease.

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Nonenzymatic antioxidants of blood in multiple sclerosis

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Abstract Free radical action has been suggested as a causal factor in multiple sclerosis. We investigated the plasma level of lipid peroxides expressed in terms of malone dialdehyde and changes in blood nonenzymatic antioxidants (glutathione, α-tocopherol, retinol, plasma sulfhydryl groups, and uric acid) in multiple sclerosis patients with exacerbation or in remission, including a group treated with β-interferon. The malone dialdehyde level was increased by 38% (n.s.) during exacerbations. The blood concentration of oxidized glutathione was likewise elevated ($P < 0.05$), while the ratio of plasma α-tocopherol to cholesterol plus triglyceride was decreased ($P < 0.001$). These changes suggest increased free radical production and consumption of the scavenger molecules during the active phase of the disease. Blood reduced glutathione level was increased ($P < 0.01$) during exacerbation and remission as well. The rise in this thiol is likely to be a compensatory mechanism defending the cells from further oxidant injuries. β-Interferon increased plasma α-tocopherol levels ($P < 0.001$) but not the lipid corrected α-tocopherol value. Other parameters were not influenced by the drug.

Key words Glutathione · β-Interferon · Malone dialdehyde · Retinol · A-Tocopherol

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of unknown origin. The risk factors for MS include genetic susceptibility and environmental influences. Mickel [33] proposed that a lipid peroxidation disturbance caused by free radicals is involved in the breakdown of the myelin sheath. Since then several studies have demonstrated the role of increased free radical production and/or a decreased antioxidant defense in the central nervous system (CNS) as causal factors of MS [5, 20, 23, 26].

While the principal site of pathology is the CNS, the lipid status and the membrane properties in the platelets and erythrocytes in the peripheral blood are also altered [14, 27]. Increased lipid peroxide levels have been observed both in the cerebrospinal fluid and in the blood of MS patients [20, 36]. Catalase, superoxide dismutase, glutathione peroxidase, and glucose-6-phosphate dehydrogenase, which may protect cell membranes from peroxidative reactions, display different activities in the erythrocytes of patients and controls [19, 21, 22, 36, 40, 43, 52]. The blood also contains nonenzymatic antioxidants, but data on their role in the pathomechanism of MS are scarce. Lack of sufficient vitamin A and E in the diet has been suggested to be a risk factor for the onset of the disease [48]. However, other studies have found that the plasma levels of these vitamins are similar in MS patients and in controls [49, 50].

The primary defense of blood against reactive oxygen species (ROS) is the glutathione redox system of the erythrocytes. In addition to protecting the host cell, erythrocyte glutathione (GSH) can also efficiently defend other tissues (e.g., lung, liver) [41, 47]. The protective mechanism by GSH results in an increased formation and subsequent translocation into plasma of oxidized glutathione (GSSG).

The plasma level of GSSG provides a sensitive index of whole body oxidative stress [1, 2]. Other, radical-scavenging antioxidants in the blood include plasma free sulfhydryl groups (SH groups), α-tocopherol, retinol, and uric acid. These scavenger molecules function individually at their own sites but may also act cooperatively or in a synergistic way to afford appropriate protection against oxidant attacks.

The present study examined the balance between the plasma concentrations of lipid peroxides and blood nonenzymatic antioxidants (GSH, α-tocopherol, retinol, plasma SH groups, and uric acid) in relation to the clinical state of MS patients. Furthermore, we were interested to establish whether β-interferon therapy, which has been shown to induce positive clinical results in MS [44], has any effect on the tested parameters.

Patients and methods

Patients

Twenty-five patients with relapsing-remitting MS were included in the study. The diagnosis of definite MS was confirmed according to the clinical and laboratory diagnostic criteria of the Posers Committee [37]. While magnetic resonance imaging (MRI) is regularly used in diagnosis, for this study the patients were rated by a standardized neurological examination. As ongoing disease activity has been observed on serial MRI scans in clinically stable patients, MRI was suggested to provide a more complete measure of disease activity than clinical evaluation alone. However, Smith et al. [42] have observed a significant association between periods of clinical worsening and MRI findings, including increase in total number, number of new lesions, and total area of gadolinium enhancement. Their finding of a significant correlation between increased MRI activity and a more active clinical disease has also been confirmed by more recent studies [12, 13, 32, 53].

The patients were divided into the following three groups based on their neurological findings (Table 1): (a) the exacerbation group, MS patients during an attack; (b) the remission group, MS patients during an attack free, stable period; and (c) the remission+IFN group, MS patients without clinical activity under long-term β-interferon treatment (Betaseron, 8 M IU subcutaneously every second day for the past 2 months). The patients had received neither steroid therapy nor vitamin supplementation during the 3 months preceding the investigation, and none were smokers. In the exacerbation group none of the patients had had more than two relapses, and the average time between the appearance of new neurological signs and blood collection was 3 days (1–3). Individuals with lower back pain served as neurological controls. Blood was always collected while fasting during the morning hours. Values affected by age or sex (malone dialdehyde-bis-(diethyl acetal) (MDA), retinol, α-tocopherol, cholesterol, triglyceride, uric acid) were adjusted accordingly.

The study was approved by the Human Investigation Review Board of the University, and informed consent was obtained from each patient participating in the study.

Chemicals

Glutathione reductase, 5,5'dithio-bis-2 nitrobenzoic acid (DTNB), *N*-ethylmaleimide, reduced nicotinamide adenine dinucleotide phosphate (NADPH), 2-thiobarbituric acid, retinol, retinol acetate, α-tocopherol, and α-tocopherol acetate were purchased from Sigma (St. Louis, Mo., USA). MDA was from Schuchardt (Munich, Germany). Sephadex G-10 was obtained from Pharmacia (Uppsala, Sweden). All other chemicals were of reagent grade.

Lipid peroxides

Plasma lipid peroxides were assayed according to the method of Wong et al. [51] and were expressed in terms of MDA. The method is based on the reaction between MDA and thiobarbituric acid, which gives rise to a MDA–thiobarbituric acid adduct. The amount of the adduct was quantified by HPLC (column: 3.9 mm × 30 cm μBondapak C18 (10 μm, Waters-Millipore, Milford, Mass., USA), mobile phase: 400 ml methanol diluted to 1000 ml with 50 mM potassium phosphate buffer, flow rate: 2 ml/min) and spectrophotometric detection at 532 nm (Pharmacia Variable Wavelength Monitor).

Reduced and oxidized glutathione

The concentrations of total and oxidized glutathione in whole blood hemolysate were measured by combining previously accepted standard methods [35]. The action of DTNB and NADPH in the presence of glutathione reductase results in a reaction cycle, the rate of which depends on the total concentration of glutathione recorded spectrophotometrically at 412 nm during the first 6 min. As the assay responds to both GSH and GSSG, GSSG must be determined separately after alkylation of GSH with *N*-ethylmaleimide. Separation of GSSG and *N*-ethylmaleimide was achieved by gel filtration with Sephadex G-10. The concentrations of the thiols were expressed with reference to hemoglobin (Hb) determined by the cyan methemoglobin method.

Plasma-free SH groups

The concentration of SH groups was determined spectrophotometrically at 412 nm [25]. The protein content of plasma samples was measured using the method of Lowry et al. [30].

Retinol and α-tocopherol

Heparinized plasma samples stored at −70°C were used for the analysis. Retinol and α-tocopherol were determined by using the

Table 1 Data on MS patients and controls

	Control group ($n = 9$)	Exacerbation group ($n = 7$)	Remission group ($n = 12$)	Remission+IFN group ($n = 6$)
Age (years)	46.1 ± 11.3	34.6 ± 4.4	37.7 ± 7.2	37.7 ± 6.8
Men/women	6/3	5/2	9/3	4/2
Duration of disease (years)	–	2.33 ± 1.19	3.42 ± 1.71	3.36 ± 1.52
Disability score	–	4.55 ± 0.56	3.21 ± 1.15	3.40 ± 0.75

Table 2 Plasma concentration of MDA, protein, retinol, α-tocopherol, triglyceride, cholesterol, and uric acid in controls and MS patients

	Control group ($n = 9$)	Exacerbation group ($n = 7$)	Remission group ($n = 12$)	Remission+IFN group ($n = 6$)
MDA (μmol/l)	1.32 ± 0.15	1.82 ± 0.21	1.66 ± 0.15	1.47 ± 0.15
Protein (g/l)	68.44 ± 2.63	63.91 ± 1.81	65.56 ± 1.67	67.17 ± 2.22
Retinol (μmol/l)	2.02 ± 0.55	2.00 ± 0.50	2.68 ± 0.38	2.58 ± 0.34
α-Tocopherol (μmol/l)	29.50 ± 2.33	15.65 ± 2.58*	34.88 ± 2.26	39.88 ± 4.23**
Triglycerides (mmol/l)	1.09 ± 0.12	0.84 ± 0.11	1.22 ± 0.13	1.34 ± 0.11
Cholesterol (mmol/l)	5.16 ± 0.30	5.18 ± 0.61	5.98 ± 0.34	5.86 ± 0.38
Uric acid (μmol/l)	247 ± 17	251 ± 22	238 ± 19	240 ± 21

* $P < 0.001$, exacerbation group vs. all the other groups
** $P < 0.001$, remission + IF group vs. control and exacerbation groups

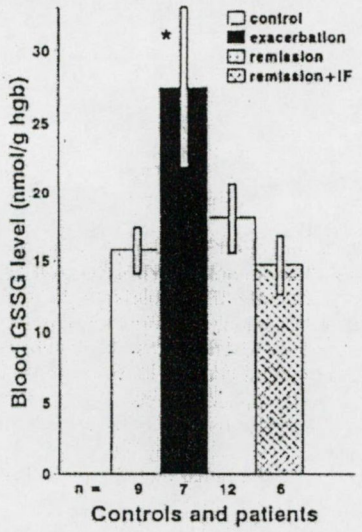

Fig. 1 Plasma GSSG concentration in controls and MS patients. In the exacerbation group the plasma GSSG concentration was significantly higher than in all other groups (*$P < 0.05$)

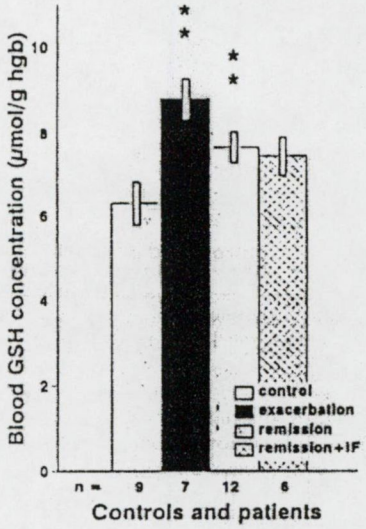

Fig. 2 Plasma GSH concentration in controls and MS patients. The GSH level was significantly higher in patients with exacerbation and in patients in remission than in controls (**$P < 0.01$)

method of Catignani and Bieri [6]. Briefly, 100 μl plasma was deproteinized with ethanol that contained the internal standards (retinyl acetate and α-tocopheryl acetate). Lipids were extracted by addition of hexane. A portion of the hexane phase was evaporated, and the residue was dissolved in diethyl ether and methanol. The amount of each vitamin was quantified by HPLC [column: C 18 Spherisorb ODS 2 (5 μm packing), 40 × 250 mm, Pharmacia, mobile phase: 95% (v/v) methanol in water, flow rate: 2.5 ml/min] and UV detection at 280 nm. The concentrations of α-tocopherol were expressed with reference to plasma cholesterol plus triglyceride [46].

Uric acid

The concentration of uric acid was measured in trichloracetic acid extracts of blood by the method of Harkness et al. [16]. Quantitative determination was made by HPLC [column: Spherisorb ODS2 (5 μm packing), 40 × 250 mm, Pharmacia, mobile phase: 0.01 M potassium phosphate buffer containing 1% (v/v) methanol, pH 6.5, flow rate: 1 ml/min] with UV detection at 280 nm.

Statistical analysis

All data are expressed as mean ± standard error. One-way analysis of variance was followed by the least-significant-difference test to determine significant differences between groups. A P value less than 0.05 was considered statistically significant.

Results

The plasma concentration of MDA was 38% higher in the exacerbation group than in the control group (Table 2), but this rise was not statistically significant.

Blood GSSG concentration was significantly higher ($P < 0.05$) in patients with exacerbation than in all other groups (Fig. 1). Blood GSH level was higher ($P < 0.01$) both in patients with exacerbation and in patients in remission than in the controls (Fig. 2).

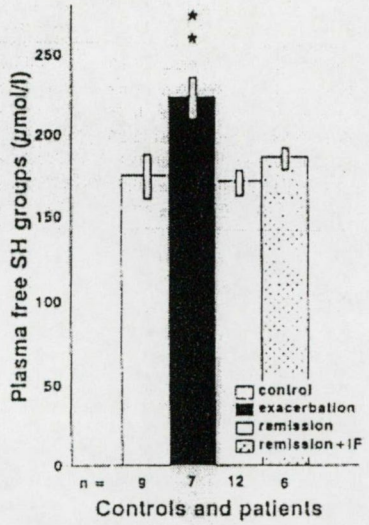

Fig. 3 Plasma SH group concentration in controls and MS patients. Patients with exacerbation had a significantly higher concentration of SH groups than all other groups (**$P < 0.01$)

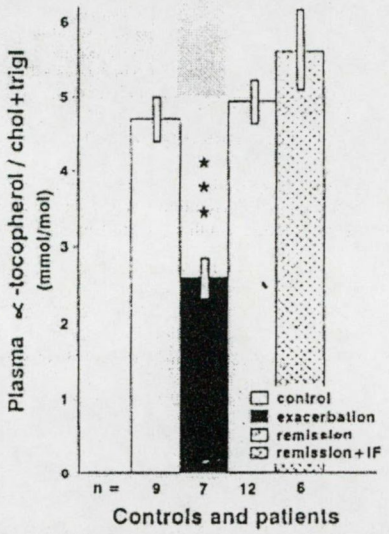

Fig. 4 Ratio of plasma α-tocopherol to cholesterol plus triglyceride in controls and MS patients. Patients with exacerbation had a significantly lower ratio than all other groups (***$P < 0.001$)

The level of plasma-free SH groups was significantly higher ($P < 0.01$) during exacerbation than in the other groups (Fig. 3).

No significant difference of the retinol level appeared between the tested patients and controls (Table 2). Both the plasma α-tocopherol concentration (Table 2) and the ratio of α-tocopherol to cholesterol plus triglyceride (Fig. 4) were significantly lower ($P < 0.001$) during exacerbation. β-Interferon increased the plasma α-tocopherol level ($P < 0.001$) but not the lipid corrected α-tocopherol value compared to controls. Cholesterol and triglyceride levels did not differ significantly between the groups (Table 2).

There was no significant difference in plasma uric acid between patients and controls (Table 2).

Discussion

In the present study, the oxidation of GSH, i.e., the elevation in GSSG concentration, and the simultaneous decrease in α-tocopherol level in the blood of MS patients provided evidence of an increased generation of ROS in the active phase of the disease. However, the formation of lipid peroxides were not significantly enhanced during exacerbation.

Both glutathione and α-tocopherol are potent inhibitors of lipid peroxidation. While the role of GSH in this process is primarily, if not solely, to prevent the initiation of radical formation, α-tocopherol inhibits the propagation of the chain reaction [28]. The slight rise in the MDA concentration, in contrast to the marked changes in the GSSG and α-tocopherol levels, in the patients in relapse suggests that the free radical generation was counteracted to a significant extent by the antioxidant defense mechanisms. It is to be noted that the studied relapse period was the first or second relapse for these patients, and that they were at the beginning of exacerbation at the time of the investigation.

The literature data concerning the plasma MDA level in MS is controversial; both significant and also considerable, but not significant elevations, or even normal values have been reported [20, 24, 36, 43]. The differences between these results are perhaps due to differences in the current phase of the disease studied (e.g., the number of exacerbation, or the beginning or end of the relapse or remission periods).

In addition to the GSSG concentration, the GSH level was also significantly increased in the blood of the patients with exacerbation. The increase in GSH under conditions of continuous oxidant generation is likely to be a compensatory mechanism which defends the cells from further oxidant injuries [10].

Hunter et al. [19] reported that MS erythrocytes are less susceptible to hydrogen peroxide induced lipid peroxidation in vivo. They suggested that the level of intracellular GSH or another endogenous antioxidant are elevated in MS erythrocytes, and this suggestion accords well with our results. In contrast, Jensen et al. [22] found a low glutathione peroxidase activity and a low GSH concentration in MS erythrocytes. They studied clinically stable patients, but this could not be the reason for the inconsistent

results, as the GSH level was also increased during remission in our study.

Erythrocyte GSH has also been suggested to be an important component of the interorgan GSH homeostasis. Dass et al. [8] proposed a model which includes a substantial output of GSH by the kidney and the liver in red blood cells, and the extraction of GSH from these cells by those tissues that have been previously identified as sites of GSH utilization including lung, heart, gut, and brain [9]. According to this model, the elevated level of GSH in the peripheral blood of MS patients observed in the present study may reflect enhanced transportation and bioavailability of this tripeptide and its constituent amino acids to peripheral tissues including the CNS.

The increased level of SH groups in the plasma during exacerbation is most probably related to the high GSH content of the erythrocytes. The mediating vehicle between these two environments may be the membrane SH groups [29, 38].

The changes observed in the blood of MS patients indicate enhanced generation of ROS; however, it is not clear how these changes at the periphery are related to the pathology in the CNS. Free radicals are thought to play a major role in destruction of the myelin sheath. Activated macrophages are able to produce both nitric oxide and superoxide anion [34]. While NO itself displays low toxicity, and also superoxide is relatively inert, the interaction of these two radicals results in formation of the powerful oxidant peroxynitrite [4]. NO and superoxide can also release iron from ferritin [39, 45]. Ferritin, an iron-storage protein, is present in almost all cell types including astrocytes, macrophages, oligodendrocytes, and microglia [7]. The iron released from the protein bond may react with superoxide anion and hydrogen peroxide to generate hydroxyl radical. Peroxynitrite and the highly reactive hydroxyl radical oxidize DNA, proteins and initiate lipid peroxidation which in turn may lead to demyelination and neuronal damage. Free radicals may also contribute to the damage of the blood brain barrier which is an early event of MS lesions [12].

The role of NO in the pathomechanism of MS is supported by the observation that the levels of inducible nitric oxide synthase (NOS) messenger RNA are markedly higher in MS brains than in normal controls [5]. Strong inducible and constitutive NOS immunoreactivity has been found in macrophages distributed within regions of active demyelination [11]. The presence of nitrotyrosine residues has also been demonstrated in the brains of MS patients [3].

The free radicals may be involved in the induction and presumably perpetuation of the disease as well. Experimental autoimmune encephalitis (EAE) is the rodent model for MS. Repeated injection of a scavenger of oxygen radicals starting at the time of EAE induction delayed the onset and markedly reduced the severity of the disease [31]. Furthermore, all treated mice completely recovered after 40 days. In another study the use of an inhibitor of iNOS induction and scavengers of NO and peroxinitrite exerted significant therapeutic effects [17]. Treatment with high doses of uric acid (scavenger of peroxinitrite) virtually prevented clinical symptoms of EAE.

Therapeutic agents which can scavenge or prevent radical formation should also be of significant benefit to the patients. β-Interferon, which is an efficacious treatment for MS, has been shown to inhibit iNOS activity and suppress endogenous NO production [15, 18]. In the present study β-interferon induced significant elevation in plasma α-tocopherol level compared to controls. However, this can be attributed at most only partially to the decreased free radical production and concomitant vitamin sparing effect of the drug. The plasma concentration of α-tocopherol is strongly correlated with plasma lipids which exert carrier functions for the lipophilic antioxidant in blood [46]; and the lipid corrected α-tocopherol values were not markedly enhanced.

In conclusion, the present study provides evidence of peroxidative reactions in MS patients during exacerbation, and supports the role of oxidative stress in the pathomechanism of the disease.

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V.

Peer reviewed article

Experiences with interferon-beta-1b treatment in MS after three year follow-up

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Interferon-beta-1b (IFNβ-1b) was the first drug which has been proven to decrease the number of attacks by 34% in relapsing-remitting multiple sclerosis (MS).

The aim of this open label, observational phase IV study was to evaluate the effect of IFNβ-1b on relapse rate in a three-year follow-up. The data of the enrolled patients in the two years prior to the treatment (1995–96) served as control. The trial was carried out between 1996 and 1999. 31 patients with definite MS received 8M IU IFNβ-1b sc. every other day. The relapse rate, the duration of hospitalisation and the steroid needs for treatment of relapses were calculated. Statistical analysis was made by one-way ANOVA. At baseline the mean age was 37 ± 8 years, the mean EDSS score was 1.8 ± 1.2 and the mean duration of the disease was 4 ± 4 years. Before treatment the annual relapse rate was 1.3, while during the treatment it decreased to 0.3 (Table 1). The relapse rate was reduced by 77% compared to pre-study values ($p < 0.001$). Before starting the therapy the patients spent 16.0 ± 2.5 days in the hospital annually. In the three years of IFNβ-1b the mean time of hospitalisation decreased by 84% ($p < 0.001$). In the two years preceeding IFNβ-1b therapy 5.7 ± 1.9 grams of methylprednisolone (MP) / patient were needed for treatment of relapses. During IFNβ-1b therapy MP needs were reduced by 75% ($p < 0.001$).

Using the patients as their own controls is a methodological problem in MS, taking into account the more or less unpredictable course and also the fact that patients initiating treatment may do so in an active phase of the disease with consecutive spontaneous regression to the mean. Nevertheless, the magnitude of the change observed in this study supports a positive effect of IFNβ-1b on the disease under everyday practice conditions.

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Table 1

Differences observed during the 3 years of IFNβ-1b treatment (mean ± SD).

years	-2	-1	+1	+2	+3
no. of relapses / patient	1.0 ± 0.5	1.6 ± 0.6	0.4 ± 0.5	0.4 ± 0.4	0.2 ± 0.4
days of hospitalisation / patient	14 ± 10	18 ± 11	3.5 ± 6.8	3.1 ± 5.2	0.7 ± 2.1
need of steroid (g) / patient	4.3 ± 2.8	7.0 ± 2.5	1.7 ± 2.9	1.7 ± 2.4	0.6 ± 1.6

Reference

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VI.

EREDETI KÖZLEMÉNYEK

Liquordiagnosztikai vizsgálatok sclerosis multiplexes betegekben

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A Poser-féle kritériumrendszer szerinti klinikai és laboratóriumi vonatkozásban definitív sclerosis multiplex diagnózis megállapításához nélkülözhetetlen a liquor cerebrospinalis analízise. A szerzők célja a sclerosis multiplex diagnózis felállításához az általuk a Charcot alapítvány javaslata szerint végzett liquorvizsgálati módszerek ismertetése. A liquoranalízis során fontos, hogy elkülönítsük a normál mennyiségű, a lokálisan szintetizálódó és a sérült vér-liquor gáton keresztül jutó immunglobulinokat. Az általános gyakorlat szerint liquor és szérum minták párhuzamos feldolgozása történik, mennyiségi és minőségi fehérjeanalízis során vizsgálataikban. Lézer nefelometriával 37 klinikailag sclerosis multiplexes betegnél meghatározták az albumint és a G, A, M immunglobulinokat. Emelkedett IgG indexet találtak 76%-ban, amely lokális IgG szintézisre utal, és ez egy lehetőség a humorális immunválasz bizonyítására. Az albuminhányados, amely a vér-liquor gát integritásának vizsgálatára alkalmas, a referenciatartományon belül volt. Minőségi fehérjeanalízist végeztek agaróz-gél elektroforézissel és izoelektromos fókuszálással. Agaróz-gél elektroforézissel 68%-ban, míg izoelektromos fókuszálással 91%-ban igazolódott oligoklonális gammopatia. Összehasonlítva a kétfajta minőségi fehérjeanalízist, az izoelektromos fókuszálás érzékenyebbnek bizonyult az oligoklonális csíkok kimutatásában, amit az irodalmi adatok is alátámasztanak.

Kulcsszavak: liquor cerebrospinalis, sclerosis multiplex, lézer nefelometria, liquor/szérum albumin, IgG index, agaróz-gél elektroforézis, izoelektromos fókuszálás (IEF), oligoklonalis gammopatia (OGP), vér-liquor gát (VLG)

Analysis of cerebrospinal fluid in patients with multiple sclerosis. The diagnostic criteria postulated by Poser necessitate clinical and laboratory CSF analysis for establishment of the diagnosis of definitive multiple sclerosis. The present paper reports methods for CSF examinations relating to multiple sclerosis with regard to the examinations suggested by the Charcot Foundation. In the course of CSF analysis, it is important to discriminate between the immunoglobulins present in normal amounts, those synthesized locally in pathological quantities and those penetrating across the damaged blood-CSF barrier. Normally, a parallel assay of CSF and serum specimens is carried out in the course of quantitative and qualitative protein analysis. In 37 patients with clinical multiple sclerosis, we determined the albumin and the immunoglobulin classes IgG, IgA and IgM, using laser nephelometry. An elevated IgG index was found in 76% of the cases, which points to local IgG snythesis and might be proof of the humoral immune response. The albumin quotient, which is suitable for examination of the integrity of the blood-CSF barrier, was within the reference range. Qualitative protein analysis was performed by means of electrophoresis on agarose-gel and isoelectric focusing. Agarose-gel electrophoresis revealed oligoclonal gammopathy in 68%, in contrast with the 91% demonstrated by isoelectric focusing. Comparison of the two kids of qualitative protein analyses indicated that isoelectric focusing was more sensitive for the detection of oligoclonal bands, in support of the literature finding.

Key words: cerebrospinal fluid, multiple sclerosis, laser nephelometry, CSF/serum albumin quotient, IgG index, Agarose-gel elektrophoresis, isoelectric focusing, oligoclonal gammopathy, blood-CSF barrier

Az SM liquordiagnosztikai analízisénél általánosan elfogadott alapelv, hogy a szérum, valamint a liquor összetevőit egymással összehasonlítva értékeljük, hiszen a vér-liquor gát funkcionális állapota jelentősen befolyásolhatja az intrathecalisan mért értékeket. A hazai adatok szerint a szétválasztott fehérjefrakciók kimutatásában a mai napig a legtöbb laboratóriumban használatos eljárás az 1972-ben *Kerényi és mtsai* által kidolgozott agaróz-gél elektroforézis, ezüst festéssel (14). A Mancini-féle radiális immundiffúzióval (19) meghatározhatjuk a liquorban és a szérumban az albumin, az IgG, IgM és IgA koncentrációt. Hazánkban az albumin és az IgG vonatkozásában a metodikát a Pécsi Orvostudományi Egyetem Idegklinikájának munkacsoportja standardizálta és módosította (15). Az IgM és IgA mérési módszerét az Uzsoki utcai Kórház Neuroimmunológiai Laboratóriumában dolgozták ki (2). Az izoelektromos fókuszálás liquordiagnosztikában történő alkalmazásakor kiemelkedő szerepe volt az OPNI Kémiai Laboratóriumának. A Charcot-alapítvány öt munkamegbeszélést szervezett 1994-ben 12 európai, akkreditált liquordiagnosztikai centrum részvételével, hogy a sclerosis multiplex diagnózisához elengedhetetlenül szükséges, ún. esszenciális tesztre és kiegészítő vizsgálatokra, valamint egyéb, lehetséges labo-

Rövidítések: SM = sclerosis multiplex; Qalb = liquor/szérum albumin; IEF = izoelektromos fókuszálás; OGP = oligoklonális gammopathia; VLG = vér-liquor gát

ratóriumi vizsgálatokra javaslatot tegyenek (1). Az SM-es beteg liquorának vizsgálatakor legfontosabb feladat a humorális immunválasz bizonyítása.

A Charcot-alapítvány esszenciális tesztként a humorális immunválasz igazolására legérzékenyebb metodikának az izoelektromos fókuszálást jelölte meg. A sclerosis multiplex diagnózisához a következő *kiegészítő* teszteket javasolták:
- a vér-liquor gát integritásának vizsgálata;
- az IgG index meghatározása;
- a liquor sejtszám mérése.

Választható tesztként a következők alkalmazhatók:
- IgM meghatározás;
- IgA meghatározás;
- a szabad kappa és lambda könnyű láncok kimutatása;
- MBP koncentrációjának mérése, továbbá rubeola, morbilli és varicella zoster antitestek lokális szintézisének meghatározása.

A Szent-Györgyi Albert OTE Neurológiai Klinikai Liquordiagnosztikai Laboratóriumában 1996-ban 37 esetben vizsgáltuk meg klinikailag sclerosis multiplexes tüneteket mutató betegek liquorát és szérumát. Minden esetben történt rutin liquorvizsgálat, melynek során összfehérjét mértünk és sejtszámot számoltunk. A liquorfehérjék mennyiségi analízisét lézer nefelometriával végeztük. Meghatároztuk az albuminhányadost, IgG, IgM, IgA indexeket. A minőségi fehérjeanalízis során pedig agaróz-gél elektroforézist és izoelektromos fókuszálást (IEF) végeztünk párhuzamosan, és összehasonlítottuk e két vizsgálat érzékenységét oligoklonalis gammopathiára (OGP) nézve. Jelen összefoglalás célja, hogy ismertessük a nemzetközi álláspontot a sclerosis multiplex liquordiagnosztikáját illetően és beszámoljunk ezen kritériumok alapján végzett vizsgálataink eredményeiről.

Betegek és módszerek

Laboratóriumunkban 1996-ban a Poser-féle kritériumrendszer szerint (21) 37 klinikailag SM-es beteg liquor- és szérum-fehérjeanalízise történt. A betegek átlagos életkora: 37,08 év (20–67 év között), a férfiak és nők közötti arány 1 : 3.

Az összes páciensnél a liquor makroszkópos vizsgálata után meghatároztuk a liquor sejtszámot Fuchs-Rosenthal kamrában. A talált sejtek százalékos értékelését Sayk-féle ülepítő kamrában végzett ülepítés után festett kenetben végeztük.

A liquorfehérjék mennyiségi meghatározása lézer nefelométerrel történt. A DOSASCAT nephelométerrel (Dosatec GmbH, München) két programban mértünk: az egyik egy általános összfehérje meghatározás, a reagens 40%-os triklórecetsav (TCA Best. Nr. 8005 Dosatec GmbH). A másik módban nagy intenzitású lézerfénnyel megvilágítva a szuszpendált antigén-antitest komplexeket, az IgG, IgM, IgA mennyiségi meghatározását egy speciális program segítségével, Reiber metódus szerint (22) a végpont-meghatározással végeztük. A mérés során párhuzamosan mérünk szérum és liquor mintákat, a szérumot IgG, IgA, IgM meghatározásnál 1 : 400, albumin meghatározásnál 1 : 2000-ben hígítjuk fiziológiás konyhasóoldattal. A liquor esetében 800 mg/l összfehérjéig az IgG, IgA, IgM meghatározásnál a liquort hígítatlanul mérjük be, albumin meghatározásnál 1:10 arányban hígítunk. (Reagensek: RA A-HU albumin Q032805, RA A-HU IgG Q033105, RA A-HU IgA Q033205, RA A-HU IgM Q033305, Dilution buffer S200530, Human serum protein calibrator X090801, Human serum protein low control X093901, Human serum protein high control X094001, DAKO, Glostrup).

A liquorfehérjék minőségi analízisét agaróz-gél elektroforézissel és izoelektromos fókuszálással végeztük. Agaróz-gél elektroforézis során benzinnel, ill. petroléterrel hűtött Kerényi-féle elektroforézis berendezést használtunk. A fehérjefrakciók feltüntetésére ezüst festést alkalmaztunk (14, 16). A minőségi fehérjeanalízist izoelektromos fókuszálással *Keir* szerint (13) végeztük. Az IEF során liquor és szérum mintákat párhuzamosan alkalmazva 1500 Volton futtatunk, amit préfókuszálás előz meg 500 Volton (MULTIPHOR II, Pharmacia LKB), az általunk használt futtató közeg, 0,5 mm-es agaróz-gél (Agarose IEF, 17-0468-01, Pharmacia, Uppsala), amely pH 3–10 tartományú ampholytet (Pharmalyte, 17-0456-01, Pharmacia, Uppsala) tartalmaz. A liquort hígítatlanul mérjük be, a szérumot 1 : 400 arányban 0,9%-os NaCl-dal hígítjuk. Az elválasztandó fehérjék izoelektromos pontjuknak megfelelően fognak elhelyezkedni. Az oligoklonalis IgG csíkok a pH 7,0–9,3-es tartományban 7–15 diszkrét csík formájában tehetők láthatóvá ezüstfestéssel, illetve megbízható kimutatásuk IgG specifikus antitest festéssel lehetséges.

Eredmények

A Poser kritérium alapján vizsgált 37 klinikailag SM-es betegnél végzett lézer nefelometriás vizsgálatoknál az intrathecalis immunglobulin-szintézis 28 esetben igazolódott. Minden esetben emelkedett az IgG index, 12 betegnél az IgM index, 6 betegnél az IgA index is nagyobb volt. 7 betegnél az IgG, IgM, IgA indexek normálértéket mutattak. Két esetben vér-liquor gát károsodást találtunk (*1. táblázat*).

1. táblázat: Intrathecalis immunglobulin-szintézis mennyiségi analízissel nyert adatai

	Esetszám	%
IgG index ↑	28/37	76
IgM index ↑	12/28	
IgA index ↑	6/28	
IgG, M, A indexek normál értékűek	7/37	19
VLG károsodás	2/37	5

A liquor/szérum albumin (Qalb) átlagértéke $4{,}95 \times 10^{-3}$ a referenciatartomány között található. Agaróz-gél elektroforézissel 25 esetben oligoklonális gammopatiát (OGP), 3 esetben diffúz gamma-szaporulatot, 1 betegnél keverék típusú ferrogramot láttunk, 8 beteg liquora normális fehérjeeloszlást mutatott.

Az izoelektromos fókuszálás során, a beteg liquorát és szérumát párhuzamosan futtatva, 34 esetben oligoklonális csíkokat találtunk a liquorban a pH 7,0–9,3-as

2. táblázat: Minőségi fehérjeanalízis eredményei

	Agaróz-gél elektroforézis (%)	Izoelektromos fókuszálás (%)
OGP	25/37 (68)	34/37 (91)
Normális fehérjeeloszlás	8/37 (21)	3/37 (9)
Diffúz gamma-szaporulat	3/37 (9)	–
Keverék típusú ferrogram	1/37 (2)	–

régióban. Egy betegnél a szérumban és a liquorban is OGP volt látható ebben a pH tartományban, 3 esetben normális fehérjeeloszlást észleltünk az IEF során *(2. táblázat).*

A liquor fehérvérsejtszám 32/37 esetben (86%) volt normális, 4/37 esetben (11%) a sejtszám több volt, mint 4/µl, és 1/37 (3%) betegnél volt több, mint 35/µl.

Megbeszélés

A Charcot-alapítvány kiegészítő tesztként javasolja a vér-liquor gát integritásának vizsgálatát, valamint az IgG index meghatározását. A liquor és szérum albumin koncentráció és a liquor/szérum hányadosa jól tükrözi a vér-liquor gát (VLG) állapotát (18). Az albumin egy globuláris fehérje, molekulasúlya 69 000. Az albumint csak a máj szintetizálja, tehát csak a szérumból kerül a liquorba, mennyisége nő a vér-liquor gát károsodása esetén. A liquor albumin/szérum albumin kortól függő, dimenzió nélküli hányados. A lumbalis liquor első 10 ml-ében 15 év alatt 5×10^{-3}, 16–40 év között $6{,}5 \times 10^{-3}$, 40–60 év között 8×10^{-3}, 60 éven túl $8–9 \times 10^{-3}$ (4). Vizsgálataink során 1996-ban 37 SM-es betegnél az albuminhányados átlagértéke $4{,}95 \times 10^{-3}$, ami hasonló a nemzetközi irodalmi adatokhoz (5, 6, 27, 28, összefogl. 3).

Az IgG index emelkedése egy lehetőség a humorális immunválasz bizonyítására SM-ben. A lokális IgG szintézist az IgG index-szel adjuk meg. Eredményeink azt mutatták, hogy 76%-ban volt emelkedett az IgG index. Ez megegyezik az irodalomban ismert adatokkal, miszerint SM-es betegek 70–90%-ában található lokális IgG szintézis a liquorban (17). Tourtelotte központi idegrendszeri IgG szintézist mutatott ki SM-es betegek 92%-ában agaróz-gél elektroforézissel (28).

A humorális immunválasz igazolására másik lehetőség az oligoklonális csíkok kimutatása a liquorban. Agaróz-gél elektroforézissel 68%-ban találtunk oligoklonális gammopatiát és 8%-ban diffúz gammaglobulinszaporulatot. Kerényi és mtsai 1975-ben SM-es betegek 70–90%-ában mutatott ki szubfrakcionált gamma-típusú ferrogramot a liquorban (16). E technikának érzékeny pontja az egyenletes hűtés, ami a szubfrakciók szétválásához szükséges, valószínűleg ennek a hiánynak tudható be, hogy 8%-ban találtunk diffúz gamma-globulin szaporulatot.

Az oligoklonális csíkok igazolása legérzékenyebben izoelektromos fókuszálással detektálhatók, a Charcot-alapítvány esszenciális tesztként jelöli meg az SM liquordiagnosztikájában (1). Valamennyi SM-es betegnél végeztünk IEF-t és 91%-ban találtunk a liquorban oligoklonális IgG-t. Hasonló adatokat találtak *Mehta és mtsa* (20), *Chu és mtsai* (7) 95%-ban mutattak ki oligoklonalitást sclerosis multiplexes betegek liquorában. Izoelektromos fókuszálással sem igazolódott OGP 9%-ban. Ez annak tudható be, hogy első attackos SM-es betegekről van szó, és a betegség korai szakaszában végzett lumbalpunctio negatív liquorleletet eredményezhet.

Összehasonlítva az agaróz-gél elektroforézist és az IEF-t saját anyagunkban, azt tapasztaltuk, hogy az izoelektromos fókuszálással sokkal érzékenyebben detektálhatók az oligoklonális csíkok, amit az irodalom is alátámaszt (24). Az IEF fokozott érzékenységű technika, rutinszerűen megkülönbözteti az oligoklonális csíkokat a definitív SM-es betegek 90%-ában, a valószínű SM-es betegek 40–60%-ában és lehetséges SM-ben 20–30%-ban (8–11, összefogl. 3).

Az IgM mennyiségi meghatározása liquorban, az IgM-index kiszámítása választható teszt az SM diagnózisában. Vizsgálataink során 37%-ban találtunk emelkedett IgM indexet SM-es betegeknél. *Kaiser* (12), *Sharief és mtsa* (23) és *Sindic és mtsai* (25) intrathecalis IgM-termelést találtak – technikától függően – az SM-es betegek 30–60%-ában. Kevésbé értékes információ az SM diagnózisában, klinikai jelentősége abban van, hogy a betegség progressziójával az intrathecalis IgM értéke csökken, míg a kórkép korai szakaszában gyakrabban mutat emelkedett értéket. Az IgA analízise is választható teszt, azonban mind mennyiségi, mind minőségi technikákkal végzett vizsgálatok kisértékűek a laboratóriumi vonatkozásban határozott SM-ben.

Az egyéb választható tesztek (MBP mérése rubeola, morbilli, és varicella zoster antitestek lokális szintézisének meghatározása) pedig rutinszerűen nem alkalmazhatók, elvégzésük kutató laboratóriumokban javasolt.

Lényeges hangsúlyozni, hogy a mai napig nincs az SM-re kizárólagosan jellemző laboratóriumi lelet, célszerű ezért a lehető legtöbb, a diagnózis valószínűségét erősítő, a terápiás beavatkozások megválasztását segítő vagy a betegség prognózisára utaló adat nyerése.

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VII.

A relapszus-remisszió kórformájú sclerosis multiplexes betegek kezelése interferon-béta-1b-vel

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Az interferon-β-1b az első farmakon, amely relapszus-remisszió kórformájú sclerosis multiplexes betegekben a relapszusok gyakoriságának 34%-os csökkentésével, a betegség progresszióját késleltetni képes. Szerzők jelen tanulmányában a SZOTE Neurológiai Klinikán egy évig interferon-β-1b-vel kezelt 35 sclerosis multiplexes beteg vett részt. Vizsgálatuk célkitűzései: a) a relapszus rátára és a kórházi ápolási időre vonatkoztatva saját tapasztalataik összehasonlítása a nemzetközi adatokkal, b) a relapszus kezeléséhez szükséges steroid igény összehasonlítása az interferon-β kezelést megelőző év és a kezelés évének adatai alapján. Vizsgálati eredményeik alapján úgy tűnik, hogy az interferon-β-1b kezelés 77%-kal csökkentheti a relapszus előfordulását. A mellékhatásokra vonatkozóan nem tapasztaltak az irodalmi adatoktól eltérő tüneteket. Az EDSS pontszámban lényeges javulást nem észleltek, mely szintén megegyezik az irodalmi adatokkal. A kórházi ápolási idő 84%-kal, a remisszióhoz szükséges methylprednisolon igény 78%-kal csökkent. Mindezek alapján az interferon-β-1b terápiával a betegek súlyos mozgáskorlátozottsága évekig késleltethető, egy részüknek munkaképessége megőrizhető, így ezen páciensek számára biztosítható sclerosis multiplexesként is a teljes értékű emberi élet.

Kulcsszavak: interferon-béta-1b terápia, sclerosis multiplex, relapszus ráta, relapszus-remisszió

Treatment of relapsing-remitting multiple sclerosis with interferon-beta-1-b. Interferon-β-1b was the first drug found to slow the progression of relapsing-remitting multiple sclerosis, with a reported decrease in the relapse rate of up to 34%. The present study involved 35 patients treated with interferon-β-1b for one year. The aims of the study were: a) to compare the changes in the relapse rate and the number of days of hospitalization with other data, b) to compare the steroid needs required to treat relapses for one year before and in the year of interferon-β-1b treatment. Our data indicated that the relapse rate may decrease as much as 77% following the introduction of interferon-β-1b treatment. The adverse effects and the changes in the EDSS grades were similar to the published data. The duration of hospitalization decreased by 84% and the amount of methylprednisolone needed for remission by 78%. This data suggest that the impairment of the condition of the patients may be delayed considerably, while some of them can continue to work for a longer period, the standard of life of these patients therefore being more tolerable.

Key words: interferon-beta-1b therapy, multiple sclerosis, relapse rate, relapsing-remitting

Az interferonok a cytokinek családjába tartozó proteinek. Három osztályát különítjük el: interferon-α, -β, -γ. Molekulatömegük 15 és 21 ezer Dalton között van; antivirális, immunmodulátor és antiproliferatív hatásokkal rendelkeznek. Az interferon-α-t a B-lymhocyták, a „natural killer" (NK) sejtek és a makrofágok termelik. Az interferon-β-t a fibroblasztok, epithelialis sejtek, monocyták, makrofágok, míg az interferon-γ-t a T-lymphocyták és a NK-sejtek termelik. Sclerosis multiplexben (SM) mind a liquorban, mind a perifériás vérben aktivált T-helper1 (TH1) és aktivált B-sejtek találhatók. A TH1 sejtek közvetítik a késleltetett típusú hyperszenzitivitási reakciót, míg a B-sejtek a humorális immunválaszért felelősek (6). Az IFN-β-1b hatásmechanizmusa SM-ben teljes egészében nem ismert. A feltételezett hatásmechanizmus: az IFN-β immunmoduláló hatása következtében csökken az IFN-γ immunrendszert aktiváló hatása. Az IFN-β hatására csökken a TNF termelés a monocytákban, ugyanakkor növekszik az IL-10 expressziója. Az interferon-β-1b-t E. coli baktérium segítségével állítják elő, a humán interferon szerkezetétől 1 aminosavban és hiányzó glikozilálásban különbözik (1).

Az IFN-β-1b (Betaferon) volt az első olyan készítmény, amelyről placebo-kontrollált, kettősvak, multicentrikus klinikai vizsgálat igazolta, hogy relapszus-remisszió, illetve relapszus-progresszió kórformájú SM-ben 34%-kal csökkenti az exacerbációk gyakoriságát (13, 16). A vizsgálatban 372 beteg vett részt (USA és Kanada), a páciensek $^1/_3$-a placebót, $^1/_3$-a 1,6 M NE, $^1/_3$-a 8 M NE IFN-β-1b kezelést kapott subcutan, másnaponta 2 éven keresztül. A vizsgálat során értékelték az exacerbációk számát és súlyosságát, az első exacerbációig eltelt időt, az exacerbáció-mentes betegek számát, a kórházi kezelések időtartamát, valamint sorozat MRI felvételekkel az MRI-vel detektálható aktív léziók számának változását. Az IFN-β-1b hatása dózisfüggőnek bizonyult. Szigni-

Rövidítések: ANOVA = analysis of variances, EDSS = expanded disability status scale, IFN-β-1b = interferon-β-1b, TH1 = T-helper1

fikáns eredményt a placebóhoz képest a nagyobb dózis (8M NE) mutatott. Az exacerbáció-mentes betegek aránya 100%-kal volt nagyobb a placebo csoporthoz képest, csökkent az első exacerbációig eltelt idő, a kórházi ápolási idő, valamint 40%-kal csökkent az MRI-vel detektálható aktív léziók száma (11, 15). Bár a betegek funkcióromlását vizsgálva nem volt szignifikáns különbség a placebo és a kezelt csoport között, a kezelt csoportban egy javuló trendet észleltek (15). A fenti vizsgálat alapján az interferon-β-1b (IFN-β) az első farmakon, amely relapszus-remisszió kórformájú sclerosis multiplexes (SM) betegekben a relapszusok gyakoriságának csökkentésével, a betegség progresszióját késleltetni képes. Jelen tanulmányunkban a SZOTE Neurológiai Klinikáján egy évig IFN-β-1b-vel kezelt 35 SM-es beteg vett részt. Vizsgálatunk célkitűzései: a) a relapszus rátára és a kórházi ápolási időre vonatkoztatva saját tapasztalataink összehasonlítása a nemzetközi adatokkal, b) a relapszus kezeléséhez szükséges steroid igény összehasonlítása az IFN-β-1b kezelést megelőző év és a kezelés évének adatai alapján.

Betegek és módszerek

Magyarországon 1996-ban került törzskönyvezésre az IFN-β-1b. Jelen tanulmányban összefoglaljuk az IFN-β-1b kezelés-sel egy év alatt szerzett tapasztalatainkat. A SZOTE Neurológiai Klinikáján 1996 július és november között 36 beteget választottunk be a vizsgálatba. A betegek beválasztása az American Academy of Neurology és a hazai Neurológiai Szakmai Kollégium ajánlása alapján történt (10, 17) *(1. táblázat)*.

1. táblázat: Az American Academy of Neurology és a hazai Neurológiai Szakmai Kollégium javaslata az IFN-β-1b kezelésre

1. relapszus-remisszió, vagy relapszus-progresszív kórformájú SM maradványtünetekkel vagy anélkül
2. Poser szerint klinikailag és laboratóriumilag határozott, vagy klinikailag határozott SM
3. a beteg állapota: EDSS 0–5,5
4. életkor: 18–50 év
5. az utolsó két évben legalább 2 relapszus

A fenti követelményrendszer alapján 36 beteg IFN-β-1b kezelésére került sor. Betegeink *Poser* (14) szerint klinikailag és laboratóriumilag határozott SM-es betegek; 26 nő, 10 férfi. Harmincnégy beteg relapszus-remisszió, míg 2 beteg relapszus-progresszív kórformát mutatott. A páciensek átlagéletkora 36 ± 8 év, a kezdeti EDSS pontszámuk (9) 2,0 ± 1,2, betegségük átlagos időtartama 5 ± 4 év. A kezelést megelőző 2 évben összesen a 36 betegnek 97 relapszusa volt, 1995-ben 36, 1996-ban 61. A 2 év alapján kiszámított éves relapszus ráta; 1,3 ± 0,4, 1995-ben 1,0 ± 0,5, 1996-ban 1,7 ± 0,7. A kezelés során a betegek másnaponta 8 M NE IFN-β-1b-t kaptak subcutan, öninjekció formájában.

Vizsgálatunk elsődleges végpontjának tekintettük, hogy mennyiben csökkenti az IFN-β-1b kezelés a relapszusok számát, egyéves utánkövetés során, önkontrollal összehasonlítva (a beteg kezelésének egy éve (1997) és a kezelést megelőző két év (1995 és 1996) relapszus rátájának összehasonlítása). Vizsgálatunk másodlagos végpontja a tolerancia, a mellékhatások és az EDSS pontszámban bekövetkezett változások, a kórházi ápolási idő és a relapszusok során a remisszióhoz szükséges steroid igény vizsgálata volt.

A terápia megkezdésekor, majd havonta, relapszus esetén a steroid kezelés előtt és után meghatároztuk az EDSS pontszámot. A vizsgálat megkezdésekor, majd az első 3 hónapban havonta, majd 3 havonta került sor laboratóriumi paraméterek (vizelet rutin, hematológiai értékek, szérum ionok, cukor, vese- és májfunkciós próbák) ellenőrzésére. Havonta rögzítettük az általunk észlelt és a betegek által jelzett mellékhatásokat. Relapszus esetén rögzítettük relapszuskor, a steroid terápia végén, majd egyhónap múlva az EDSS pontot, kiszámítottuk a steroid igényt, valamint a kórházi ápolási időt. A statisztikai számítások „one-way ANOVA" analízissel történtek, a páronkénti összehasonlítást Bonferroni-módszerrel végeztük.

Eredmények

Egy betegünknél a kezelés első 5 hónapjában tartós láz, testszerte izomfájdalmak jelentkeztek, majd mellkasi szorító fájdalom miatt myocardialis infarctus gyanújával intenzív osztályra került. Intenzív észlelés első napján az IFN-β-1b terápiát leállítottuk. EKG, kardiológiai UH, enzimértékek acut myocardialis történés lehetőségét kizárták, IFN-β-1b elhagyását követően láza megszűnt. Intenzív észlelés 2. napján tetraparesis alakult ki, mely tünetet a betegség ismételt relapszusaként értékeltünk. Megadózisú methylprednisolon terápia hatására a beteg remisszióba került, izomfájdalma megszűnt. Ezt követően a beteg kérésére a mellékhatások miatt a további IFN-β-1b kezelést felfüggesztettük.

2. táblázat: Interferon-β-1b kezelt betegek éves relapszus száma (betegszám: 7)

Relapszus-szám	1 attack	2 attack	3 attack
Betegszám	5	1	1

Tekintettel arra, hogy egy betegünk kezelését 5 hónap után felfüggesztettük, eredményeinket a továbbiakban 35 beteg vizsgálatára vonatkoztatjuk. Összehasonlítottuk a terápia előtti 2 év alapján kiszámított relapszus rátát az IFN-β-1b kezelés alatt észlelt relapszus rátával, valamint betegeink terápia előtti és 1 év kezelés utáni EDSS pontját. Összehasonlítottuk az 1995 és 1996-os év kórházi ápolási idejét, a remisszióhoz szükséges steroid igényt, a terápia évének eredményeivel. A terápia előtti relapszus ráta 1,3 ± 0,4 volt, míg a kezelés hatására a relapszus ráta 0,3 ± 0,8-ra változott, ami azt jelenti, hogy az IFN-β-1b kezelés 35 betegünk esetén 77%-kal csökkentette a relapszus rátát, önkontrollhoz viszonyítva ($p < 0,001$). Harmincöt betegünkből 28 esetén nem észleltünk relapszust, 5 betegünknek 1 relapszusa, 1 betegnek 2 exacerbációja, 1 betegnek 3 shubja volt a vizsgálat ideje alatt *(2. táblázat)*. A kezdeti EDSS pontszám 2,0 ± 1,2 volt, egy év kezelést követően 1,9 ± 1,7 lett, statisztikailag nincs szignifikáns különbség ($p < 0,51$).

A mellékhatásokat értékelve 34 betegünk esetén tapasztaltunk az injekció beadási napján szubfebrilitást, lázat, mely panaszok 24 beteg esetében a 3. hónapra megszűntek, míg a további 10 beteg esetében huzamosabb ideig fennálltak. Harminc betegnél az injekció helyén fájdalmas bőrpír jelentkezett *(3. táblázat)*, egy beteg esetében a szúrás helyén necrosis alakult ki, ezen

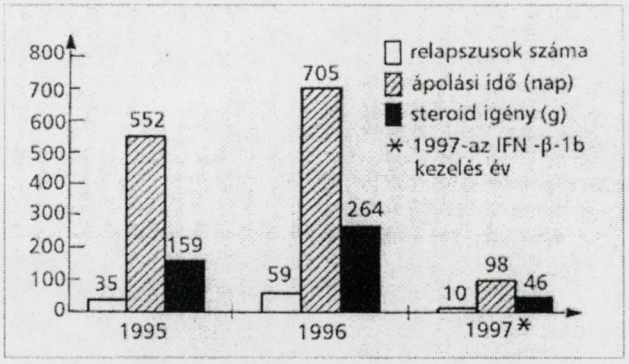
3. táblázat: Interferon-β-1b kezelés során észlelt mellékhatások (betegszám: 35)

Mellékhatás	1. hónap	3. hónap	Tartós kisebb intenzitással
Szubfebrilitás, láz	34	24	10
Lokális reakció az injekció beadási helyén	30	30	0
Necrosis az injekció helyén	1	0	0
Influenza-szerű tünetek	10	0	0
Fejfájás	3	0	0
Depresszió	3	3	0

tünetek a kezelés 3. hónapjára elmúltak. Az injekció következtében szórványosan a későbbiekben is előfordult fájdalmatlan kis kiterjedésű bőrpír jelentkezése, de ezen tünetet a betegek a kezdeti reakciókhoz képest jól tűrték. Tíz betegünknél az első hónapban influenzaszerű panaszok jelentkeztek, 3 betegünk gyakori, tenziós jellegű fejfájásról számolt be. Három betegünknél hangulati labilitás, síráskényszer jelentkezett, mely tünetek antidepresszáns adására 3 hónap után megszűntek, tartós antidepresszáns kezelést a betegek nem igényeltek. A kezelés során a laboratóriumi paraméterekben eltérést nem észleltünk. Öt betegünknél tapasztaltunk 1–2 hónapig tartó leukopeniát, ami a harmadik hónapra megszűnt. 1995-ben az IFN-β-1b-vel kezelt 35 betegünk összesen 552 napot töltött kórházban, 1996-ban a kórházi ápolási idő 705 nap volt, ami éves átlagot számítva 628,5 napot jelent. 1997-ben 98 nap volt betegeink összesített kórházi ápolási ideje. Az IFN-β-1b hatására a kórházi ápolási idő 84%-kal csökkent ($p < 0{,}001$). A relapszusok kezeléséhez a *Beck és munkatársai* által a Longitudinal Optic Neuritis Studyban alkalmazott sémát használtuk (2), azzal a módosítással, hogy ha a beteg relapszuskor észlelt EDSS pontja 0–4 között volt, akkor 3 g iv. methylprednisolon kezelést követően per os kezelésre tértünk át, ha EDSS pontszám relapszuskor több volt, mint 4, akkor 5 g iv. methylprednisolont követően tértünk át a per os kezelésre. Betegeink relapszusának kezeléséhez 1995-ben 159 gramm, 1996-ban 264 gramm methylprednisolonra volt szükség, az éves átlag 211,5 gramm. 1997-ben betegeink relapszusának kezeléséhez 46 gramm methylprednisolonra volt szükség. Az IFN-β-1b kezelés 78%-kal csökkentette a relapszusokhoz szükséges methylprednisolon igényt ($p < 0{,}001$).

Megbeszélés

Az elmúlt tíz év klinikai vizsgálatainak köszönhetően a relapszus-remisszió kórformájú SM-es betegek kezelésében a korábbiakhoz viszonyítva új stratégiát kell követnünk. A betegek terápiáját két részre bonthatjuk: az akut exacerbáció kezelésére, valamint a betegség aktivitásának csökkentésére. Az akut exacerbáció kezelésére vonatkozóan napjainkban a legelfogadottabb séma, a *Beck és munkatársai* által javasolt megadózisú methylprednisolon terápia (2). A betegség aktivitásának csökkentésére ma már négy farmakon áll rendelkezésünkre. Ezek a szerek: a β-interferonok (1a-1b), valamint a glatiramer-acetát (3, 5, 7, 14, 16). Az IFN-β-1b-n (Betaferon) kívül két IFN-β-1a (Avonex, Rebif) készítményről is igazolódott, hogy csökkenti a relapszusok gyakoriságát. Az Avonex késlelteti relapszus-remisszió kórformájú SM-es betegekben a funkcióromlás progresszióját, továbbá 18%-kal csökkenti az exacerbációk számát, valamint csökkenti a gadolínium kontrasztos T1-súlyozott MRI scaneken a léziók számát és nagyságát (4). A Rebiffel végzett klinikai tanulmányban 2 dózist alkalmaztak (22–44 mg/6–12 M NE), mindkét dózis szignifikánsan csökkentette az exacerbációk számát (29%–32%) és súlyosságát, növelte az exacerbációmentes betegek számát, csökkentette a steroid kezelések számát, mindkét dózis csökkentette az MRI-vel detektálható aktív léziók számát (3). A glatiramer-acetát (Copaxone) egy szintetikus polipeptid keverék, amelyről szintén multicentrikus, kettősvak, placebo-kontrollált vizsgálattal igazolták, hogy 29%-kal csökkenti relapszus-remisszió kórformájú SM-es betegekben az exacerbációk számát (7).



1. ábra: Az éves relapszus-szám, az ápolási napok és a steroid igény változása az INF-β-1b kezelt páciensknél

Újabb adatok vannak arra vonatkozóan, hogy az IFN-β-1b nemcsak a relapszus-remisszió formájú SM-ben hatásos, hanem a szekunder progresszív SM-ben is késlelteti a betegség progresszióját (8). Ezen klinikai tanulmányok alapján a β-interferonok és a glatiramer-acetát alkalmazásával csökkenthető a SM-es betegek súlyos mozgáskorlátozottá válása.

Saját vizsgálati eredményeink alapján úgy tűnik, hogy az IFN-β-1b kezeléssel az nem 18–34%-kal (5, 13), hanem 77%-kal csökkenthető a relapszus ráta. Hasonló eredményekről számoltak be *Harrdová és mtsai* (közlés előtt álló munka). Ezen eredményt a gyógyszer hatékonyságán túl, részint a jobban megszűrt beteganyagnak, a kezelés megkezdésekor észlelt alacsony EDSS pontnak, a viszonylag rövid (4–5 év) kórtörténetnek, továbbá a kis betegszám következményének tartjuk. Vizsgálataink felépítésével megegyező tanulmányban a shubráta 49%-os csökkenését észlelték 30 IFN-β-val kezelt páciensnél (4). A mellékhatásokra vonatkozóan nem tapasztaltunk az irodalmi adatoktól eltérő tüneteket (12). Az EDSS pontszámban lényeges javulást nem észleltünk, mely

adat szintén megegyezik az irodalmi adatokkal (13). A multicentrikus kettősvak klinikai vizsgálatok alapján az IFN-β kezelés következtében a kórházi ápolási napok, valamint a steroid igény csökkenése észlelhető (5, 13). Az irodalom konkrét, számszerű adatokat azonban nem közöl. Jelen vizsgálatunkban a kórházi ápolási idő 84%-kal, a relapszushoz szükséges methylprednisolon igény 78%-kal csökkent. A legfontosabb szempont azonban az kell hogy legyen, hogy a betegek súlyos mozgáskorlátozottsága évekig megelőzhető, a betegek egy részének munkaképessége megőrizhető, így ezen betegek számára biztosítható SM-esként is a teljes értékű emberi élet.

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VIII.

A relapszus-remisszió kórformájú sclerosis multiplex kezelése

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THERAPY OF RELAPSING-REMITTING MULTIPLE SCLEROSIS

Thanks to the multicentre, double-blind, randomized, placebo-controlled clinical trials in the last 5 years a significant progress in the therapy of relapsing-remitting multiple sclerosis was achieved. The aim of steroid therapy is to reduce the rate of acute exacerbations. The first-choise recommended therapeutical strategy is the megadosis steroid therapy according to the Longitudinal Optic Neuritis Study. Drugs are available nowaday to reduce the progression of the disease: interferon-β-1a interferon-β-1b and glatiramer-acetate. The new therapeutic possibilities provide us to slow down the progression of multiple sclerosis and to delay the worsening of patient's condition.

Az elmúlt öt év multicentrikus, randomizált, kettős vak, placebokontrollált, klinikai vizsgálatainak köszönhetően a relapszus-remisszió kórformájú sclerosis multiplex kezelésében jelentős előrelépés történt. A betegek szteroidkezelésének célja az akut exacerbáció tüneteinek és időtartamának csökkentése. Ennek érdekében az első választandó terápiás stratégia a Longitudinal Optic Neuritis Study-ban (LONS) közölt megadózisú methylprednisolon-kezelés. A betegség aktivitásának csökkentésére új hatóanyagok állnak rendelkezésre: az INF-β-1a, INF-b-1β és a glatiramer-acetát. Ezen új terápiás lehetőségekkel biztosítható a betegség progressziójának lassítása, a beteg rokkantságának megelőzése, illetve késleltetése.

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multiple sclerosis, steroid therapy, interferon-beta, glatiramer-acetate

sclerosis multiplex, szteroidterápia, interferon-béta, glatiramer-acetát

A sclerosis multiplex a mérsékelt égövön a fiatal felnőttek egyik leggyakoribb neurológiai megbetegedése. A betegség etiológiája ismeretlen. Napjainkban a legelfogadottabb teória, hogy a betegség vírusexpozíció kiváltotta autoimmun folyamat következménye *(1)*.

A betegség prevalenciája változó. Finnországban 54–91/100 000, Angliában 97–224/100 000, míg Németországban 43–131/100 000 *(2, 3)*. Adataink alapján Szegeden 65/100 000 az előfordulási arány. A sclerosis multiplex főleg a fiatal, fehér nők betegsége, a férfi-nő arány 1:2 *(4)*.

A betegség öt különböző klinikai kórformában zajlik *(1. táblázat)*. A betegek 5-15%-a a sclerosis multiplex benignus formájában szenved, ami azt jelenti, hogy 15-20 év kórlefolyás után is csaknem tünetmentes, s csak néhány exacerbáció észlelhető a kórtörténete során. A sclerosis multiplex 10-12%-os gyakorisággal primer krónikus progresszív formában zajlik. Ezen kórforma esetén jelenleg a betegség progresszióját lassító, placebokontrollált, kettős vak, multicentrikus klinikai vizsgálatokkal alátámasztott kezelési mód nem áll rendelkezésünkre. A betegek 60-80%-a relapszus-remisszió kórformát mutat. A relapszus-progresszióval járó kórforma 1-2%-os gyakoriságú, míg a betegek 5-10%-ának betegsége szekunder krónikus progresszív lefolyású *(5)*. Az utóbbi hat-hét évben a relapszus-remisszió,

1. táblázat. A sclerosis multiplex kórformáinak gyakorisága

Kórforma	Gyakoriság
Benignus	5-15%
Primer krónikus progresszív	10-12%
Relapszus-remisszió	60-80%
Relapszus-progresszív	1-2%
Szekunder krónikus progresszív	5-10%

relapszus-progresszió kórformákban több klinikai vizsgálattal igazolt kezelési mód vált ismertté, amelyek a betegség lefolyását kedvezően befolyásolják.

A betegek terápiája során célunk az akut exacerbáció kezelése, a betegség aktivitásának csökkentése, valamint a krónikus tünetek enyhítése.

Az akut exacerbáció kezelése

A sclerosis multiplex állatmodelljében, a kísérletes allergiás encephalomyelitisben (EAE) sikerült igazolni a szteroidkezelés kedvező hatását. A glükokortikoidkezelés blokkolja a kísérletes allergiás encephalomyelitis kialakulását, míg egy glükokortikoidantagonista anyag, az RU486 hatására progrediál a gyulladásos folyamat *(6)*. Patkányoknál 25 nap után hirtelen elhagyva a dexamethasonkezelést a betegség fellángolását észlelték. Azoknál az állattörzseknél, amelyek rezisztensnek mutatkoznak a kísérletes allergiás encephalomyelitisszel szemben, nagyobb mellékvesét találtak és magasabb glükokortikoidszintet regisztráltak *(7)*. Ám ha adrenalectomia után történt az immunizálás, ezek a rágcsálók ugyanúgy betegek lettek *(8)*. Sclerosis multiplexben a megváltozott hypothalamus-hypophysis-mellékvesekéreg funkciónak terápiás jelentőséget tulajdonítanak *(9, 10)*.

A *kortikoszteroidok* immunológiai hatásai sokrétűek. Redukálják a perifériás vérben lévő T-lymphocyták számát *(11)*, csökkentik a lymphocyták aktivációs stimulusra adott válaszkészségét *(12, 13)*. Visszaszorítják a citokinkiáramlást *(14, 15)*, ezáltal csökken a lymphocytaklónok kiáramlása. Gátolják a macrophagokból a tumornekrózis-faktor (TNF) felszabadulását, amely az oligodendrogliákra toxikus *(16, 17)*. Ezenkívül megakadályozzák a γ-interferon felszabadulását a lymphocytákból *(18)*. Csökkentik a MHC-receptorok expresszióját és a macrophagaktivációt *(19)*, valamint a macrophagokon és a neutrofil lymphocytákon történő Fc-receptor-expressziót, ezáltal csökkentik a sejtek citotoxikus hatását *(20, 21)*. Tartósan visszaszorítják az IgG-termelést a központi idegrendszerben, iv. kezelés után három hónappal is *(22, 23)*. A vér-agy gát átjárhatóságát csökkentik ugyan, de nem teszik teljesen átjárhatatlanná. A nagy dózisú methylprednisolon ezen túlmenően még más, nem immunmodulátor hatásokkal is bír:

2. táblázat. A szteroidterápiára vonatkozó kezelési stratégiák és ajánlások. *A legelterjedtebb kezelési stratégia napjainkban a Beck és munkatársai (26) által alkalmazott megadózisú szteroidterápia*

Szerzők	Betegek	Csoportosítás	Kezelési stratégia
Thompson és mtsai *(25)*	61 beteg	1. csoport	7 nap im. 80 E/nap ACTH 4 nap im. 40 E/nap ACTH 3 nap im. 20 E/nap ACTH
		2. csoport	3 nap iv. 1 g MP
Beck és mtsai *(26)*	389 beteg		3 nap iv. 1 g MP 11 nap p.o. 1 mg/ttkg/nap prednison
La Mantia és mtsai *(27)*	31 beteg	1. csoport	7 nap iv. 8 mg DX 4 nap iv. 4 mg DX 3 nap iv. 2 mg DX
		2. csoport	7 nap iv. 40 mg MP 4 nap iv. 20 mg MP 3 nap iv. 10 mg MP
		3. csoport	3 nap iv. 1 g MP 3 nap iv. 500 mg MP 3 nap iv. 250 mg MP 3 nap iv. 125 mg MP 2 nap iv. 62,5 mg MP
Barnes és mtsai *(28)*	80 beteg	1. csoport	7 nap 48 mg MP 7 nap 24 mg MP 7 nap 12 mg MP
		2. csoport	3 nap iv. 1 g MP

ACTH: adrenocorticotrop hormon; MP: methylprednisolon; DX: dexamethason

mérsékeli a vér-agy gátnak a demyelinizálódó területen meglévő zavarát. Kötődik a posztkapilláris venulák endothelsejtjeihez, s ezzel csökken a fehérvérsejteket az éren kívüli területekhez irányító adhéziós molekulák expressziója, és antioxidáns hatása van *(24)*. A fenti adatok alapján a szteroidok hatékonynak ígérkeznek a sclerosis multiplex akut exacerbációja esetén. A kérdés, hogy melyik készítményt és milyen dózisban alkalmazzuk.

Randomizált klinikai vizsgálatban *Thompson* és munkatársai *(25)* 61 sclerosis multiplexben szenvedő beteg relapszusát kezelték *(2. táblázat)*. Harminckét beteg három napig iv. placebo-, majd 14 napig im. ACTH-kezelésben részesült (hét napig 80 E/nap, majd négy napig 40 E/nap, három napig 20 E/nap), 29 beteg három napon át napi 1 g methylprednisolont kapott iv., majd 14 napig im. placebót. E vizsgálatban nem találtak szignifikáns különbséget a nagy dózisú methylprednisolonnal és az ACTH-val kezelt csoport között a remisszió mértékében. A három napig tartó iv. methylprednisolon-kezelés azonban a betegek számára tolerálhatóbb, hamarabb hoz létre remissziót, mint a 14

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- A sclerosis multiplexben szenvedő betegek 60-80%-a relapszus-remisszió kórformát mutat.
- A betegek terápiája során a cél az akut exacerbáció kezelése, a betegség aktivitásának csökkentése, valamint a krónikus tünetek enyhítése.
- A szteroidkezelés a betegség aktivitását nem befolyásolja, az akut tünetek enyhítésére, a relapszus időtartamának lerövidítésére alkalmas.
- Az irodalmi adatok alapján úgy tűnik, hogy a nagy dózisú methylprednisolon-kezelés a leghatásosabb a sclerosis multiplex akut exacerbációjának kezelésére, de a kis dózisú prednison-, methylprednisolon-kezelés is szóba jön.
- Klinikai vizsgálatok igazolták, hogy a sclerosis multiplex relapszus-remisszió formájában mind az INF-β, mind a glatiramer-acetát csökkenti az exacerbációk számát, és mérsékli, illetve késlelteti a betegek funkcióromlását.
- Magyarországon a négy ismertetett szer közül három, a Betaferon, az Avonex és a Copaxone törzskönyvezett.

napig tartó ACTH-kezelés, ezért a nagy dózisú methylprednisolont javasolják a betegek relapszusának kezelésére.

Beck és munkatársai *(26)* 389 opticusneuritisben szenvedő beteg követése során multicentrikus, placebokontrollált, kettős vak vizsgálattal bizonyították, hogy a három napon keresztül adott, napi 1 g iv. methylprednisolon, majd 11 napon át napi 1 mg/ttkg per os prednisonkezelés hatásos. A betegek állapotában remisszió következik be a kis dózisú prednison, valamint a placebocsoporthoz képest. A vizsgálat alapján az iv. methylprednisolonnal kezelt csoportban 57%-kal alacsonyabb a sclerosis multiplex kialakulásának valószínűsége a placebóval és a prednisonnal kezelt csoportokhoz képest.

La Mantia és munkatársai *(27)* a methylprednisolon és a dexamethason összehasonlítása céljából egy évig követtek 31 beteget. Az első csoport hét napig 8 mg dexamethasont kapott, négy napig 4 mg-ot és végül három napig 2 mg-ot. A második csoport hét napig 40 mg methylprednisolont, amit négy napig 20 mg, majd három napig 10 mg lecsengő dózis követett. A harmadik csoport megadózisú methylprednisolon-terápiában részesült, három napig napi 1 g-ot, majd háromnaponként váltakozva 500 mg-ot, 250 mg-ot, 125 mg-ot és végül két napig 62,5 mg-ot kapott. A methylprednisolon és a dexamethason hatását a remisszióra közel azonosnak találták, de a methylprednisolon-kezelést hosszabb relapszusmentes időszak követte. Figyelembe véve az egyes csoportok kis esetszámát, a statisztikai értékeléshez további vizsgálatok szükségesek.

Barnes és munkatársai *(28)* 80 beteg randomizált, multicentrikus, placebokontrollált, kettős vak vizsgálatában azt tapasztalták, hogy a 21 napig adott orális methylprednisolon és a három napig adott iv. 1 g methylprednisolon között a remisszió mértékére vonatkoztatva nincs jelentős különbség. A tanulmányban részt vett betegek közül 13 EDSS- (expanded distability status scale) *(29)* pontja 8-9 volt, e betegek többsége az intravénásan kezelt csoportba került. A vizsgálatban emiatt a két betegcsoport nem tekinthető homogénnek sem az EDSS-pontszám, sem a betegség időtartama alapján. Ezért a kis betegszámú vizsgálat eredményeiből levont következtetések a két terápia azonos értékére vonatkoztatva kérdésesek.

Az irodalomban ma sincs egységes állásfoglalás a sclerosis multiplex akut exacerbációjának terápiás stratégiáját illetően. Az első, nagyszámú és homogén betegcsoportot tanulmányozó, placebokontrollált, kettős vak multicentrikus, randomizált vizsgálat a *Beck* és munkatársai *(26)* által publikált opticusneuritis vizsgálat (LONS) volt.

Az irodalmi adatok alapján úgy tűnik, hogy a nagy dózisú methylprednisolon kezelés (1 g/nap methylprednisolon iv. 3-5 napig, majd 1 mg/ttkg/nap methylprednisolon per os) a leghatásosabb a sclerosis multiplex akut exacerbációjának kezelésére, de a kis dózisú prednison-, methylprednisolon-kezelés is szóba jön. A szteroidkezelés a betegség aktivitását nem befolyásolja, az akut tünetek enyhítésére, a relapszus idejének lerövidítésére alkalmas.

A betegség aktivitásának csökkentése

Az elmúlt tíz év klinikai vizsgálatai számos farmakonról bizonyították, hogy a sclerosis multiplex relapszus-remisszió, relapszus-progresszió kórformájában hatnak a betegség aktivitására. Ezek a szerek a β-interferonok (1a-1b), valamint a glatiramer-acetát *(30–33)*.

Interferonok

INF-β-1b

Az interferonok a citokinek családjába tartozó proteinek. Három osztályukat különítjük el: interferon-α, -β, -γ. Molekulatömegük 15 és 21 ezer Dalton között van; antivirális, immunmodulátor és antiproliferatív hatásokkal rendelkeznek. Az interferon-α-t a B-lymphocyták, a natural killer (NK) sejtek és a macrophagok termelik. Az interferon-β-t a fibroblastok, az epithelialis sejtek, a monocyták, a macrophagok, míg az interferon-γ-t a T-lymphocyták és az NK-sejtek termelik. Sclerosis multiplexben mind a liquorban, mind a perifériás vérben aktivált T-helper$_1$ (TH_1) és aktivált B-sejtek talál-

hatók. A TH_1-sejtek közvetítik a késleltetett típusú hiperszenzitivitási reakciót, míg a B-sejtek a humorális immunválaszért felelősek. Az IFN-β hatásmechanizmusa sclerosis multiplexben teljes egészében nem ismert. A feltételezett hatásmechanizmus: az IFN-β immunmoduláló hatása következtében csökken az IFN-γ immunrendszert aktiváló hatása. Az IFN-β hatására csökken a TNF-termelés a monocytákban, ugyanakkor növekszik az IL-10 expressziója *(34, 35)*.

Az IFN-β-1b *(Betaferon)* volt az első olyan készítmény, amelyről placebokontrollált, kettős vak, multicentrikus klinikai vizsgálat igazolta, hogy relapszus-remisszió, relapszus-progresszió kórformájú sclerosis multiplexben 34%-kal csökkenti az exacerbációk gyakoriságát *(3. táblázat)*. A vizsgálatban 372 beteg vett részt (USA és Kanada), akiknek egyharmada placebót, egyharmada 1,6 M NE és egyharmada 8 M NE INF-β-1b-t kapott subcutan, másnaponta, két éven keresztül. A vizsgálat során értékelték az exacerbációk számát és súlyosságát, az első exacerbációig eltelt időt, az exacerbációmentes betegek számát, a kórházi kezelések időtartamát, valamint MRI-sorozatfelvételekkel az MRI-vel detektálható aktív laesiók számának változását.

Az IFN-β-1b hatása dózisfüggő volt. Szignifikáns eredményt a placebóhoz képest a 8 M NE dózis mutatott. Az exacerbációmentes betegek aránya 100%-kal volt nagyobb a placebocsoporthoz képest, csökkent az első exacerbációig eltelt idő, a kórházi ápolási idő, valamint 40%-kal csökkent az MRI-vel detektálható aktív laesiók száma. Bár a betegek funkcióromlását vizsgálva nem volt szignifikáns különbség a placebo- és a kezelt csoport között, javuló trendet észleltek a kezelt csoportban *(30)*.

INF-β-1a

Újabb klinikai vizsgálatok zárultak le a két INF-β-1a (Avonex, Rebif) készítménnyel az elmúlt két évben *(31, 32)*. Az *Avonexről* igazolódott, hogy szignifikánsan késleltette a relapszus-remisszió kórformájú sclerosis multiplexben szenvedő betegek funkcióromlásának progresszióját olyan betegekben, akiknek EDSS-pontszáma 0–3,5 között volt, valamint 18%-kal csökkentette az exacerbációk számát (statisztikailag nem szignifikáns eredmény a feltétlenül kezelendő populációban). Csökkentette a gadolínium kontrasztos T1-súlyozott MRI-scaneken a laesiók számát és nagyságát *(31)*. A *Rebiffel* végzett klinikai tanulmányban két dózist alkalmaztak (22 és 44 mg = 6, illetve 12 M NE). Mindkét dózis szignifikánsan csökkentette az exacerbációk számát (29%, 32%) és súlyosságát, növelte az exacerbációmentes betegek számát, csökkentette a szteroidkezelések

3. táblázat. Az interferonok adagolási javaslata. *Legeredményesebben az INF-β-1b csökkenti az exacerbációk számát*

Farmakon	Javasolt adagolás	Az exacerbációk számának csökkenése
INF-β-1b (Betaferon)	másnaponta sc. 8 M NE	>34%
INF-β-1a (Rebif)	másnaponta sc. 6 M NE	29%
INF-β-1a (Avonex)	másnaponta sc. 12 M NE	32%
	hetente 1× im. 6 M NE	18%

számát, mindkét dózis csökkentette az MRI-vel detektálható aktív laesiók számát. Dózisfüggő hatást észleltek; abban a betegcsoportban, akiknek betegsége másodlagos progresszív formába mehetett át, a nagyobb dózist hatékonyabbnak találták *(32)*.

Az INF-β-1b, -1a-val végzett vizsgálatok bebizonyították, hogy az INF-β a sclerosis multiplex relapszus-remisszió formájában csökkenti a relapszusok gyakoriságát, ezáltal a betegség természetes lefolyását pozitívan befolyásolja *(30–32)*.

Glatiramer-acetát

A glatiramer-acetát *(Copaxone)* a bázikus myelinproteinben megtalálható négy aminosav (L-alanin, L-glutaminsav, L-lizin, L-tirozin) szintetikus polipeptid keveréke. *Teitelbaum (36)* vizsgálatai igazolták, hogy a glatiramer-acetát-kezelés következtében mérséklődnek a kísérletes allergiás encephalomyelitis tünetei. A glatiramer-acetáttal végzett előzetes vizsgálatok igazolták, hogy a szer nem toxikus *(37)*. A farmakon feltételezett hatásmechanizmusa sclerosis multiplexben: kompetitíven gátolja a bázikus myelinprotein vagy más autoantigén kötődését az MHC-II receptorokhoz, illetve a T-sejtekhez *(38)*. A glatiramer-acetátról 1995-ben egy kettős vak, placebokontrollált, multicentrikus klinikai vizsgálat igazolta, hogy 29%-kal csökkenti a relapszus-remisszió formájú sclerosis multiplexben az exacerbációk számát. Nem volt szignifikáns hatása a betegek funkcióromlására, de több beteg javulásának és kevesebb beteg romlásának trendje volt megfigyelhető. Mellékhatásprofilját tekintve jól tolerálható készítmény *(33)*.

Az elmúlt öt év multicentrikus, randomizált, kettős vak, placebokontrollált, klinikai vizsgálatainak köszönhetően a relapszus-remisszió kórformájú sclerosis multiplex kezelésében jelentős előrelépés történt. A betegség aktivitásának csökkentésére négy gyógyszer is rendelkezésre áll: az INF-β-1a, -1b és a glatiramer-acetát (Magyarországon a Betaferon, az Avonex és a Copaxone

törzskönyvezett) *(29-32)*. Ezen új terápiás lehetőségekkel biztosítható a betegség progressziójának lassítása, a sclerosis multiplexben szenvedő beteg rokkantságának megelőzése, illetve késleltetése.

Köszönetnyilvánítás

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IX.

Familial multiple sclerosis: case study of three affected siblings

Abstract

We report on three sisters with new-onset multiple sclerosis (MS).

The symptoms of the eldest sister began in 1993 with lower-limb weakness and paraesthesia. In 1998, she had limb weakness, nystagmus and ataxia. Magnetic resonance imaging (MRI) of the brain, the CSF examinations, and evoked potentials verified MS. The middle sister exhibited left-side optic neuritis in 1998. All findings pointed to MS. The third sister had subjective complaints such as paraesthesias and vertigo. MRI and CSF results supported the diagnosis. Both parents and all four grandparents are without neurological signs; the brain MRI examinations on the parents were negative.

The prevalence of familial MS in first-degree relatives is 5-10%, while that in twins is 20-30%. In this case environmental factors seem to play the crucial role. Although the anamnesis as concerns MS proved negative in the other family members examined here, further genetic examination of the sisters is needed.

Familial multiple sclerosis: case study of three affected siblings

Keywords: familial, multiple sclerosis, new-onset

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Objectives

In sequence of the improved genetic epidemiological tools and statistical methodology, it has become clear that multiple sclerosis (MS) is a complex trait with genetic epidemiology very similar to that of a number of other organ-specific autoimmune diseases. The susceptibility to the illness is determined by a number of largely uncharacterized genes and environmental factors. However, it is not easy to see any analogy or to postulate any particular mechanism whereby these factors exert their effects. It is therefore useful to make a careful study of the over- and under-expression of the responses to environmental exposures (1-5). It has become evident that the parents or siblings of the MS patients are often carrying the trait of the disease, however without any neurological symptom. Shared childhood and adolescence seems to have little impact on the risk of development of MS in siblings; the sequence of birth of affected individuals is more important than year of onset (1, 3, 6, 7). We met the unusual occurrence where all three sisters were suffering from MS without any MS trait regarding the parents and grandparents.

We report here on three sisters with new-onset MS. The family history (four grandparents and two parents) lack on MS or any other autoimmune or neurological disease. Relatives of MS patients are at greater risk for developing the disease than the general population, although this risk is still relatively low in absolute terms. Children of affected parents are reported, but we have not found any other report on affected siblings without any familial MS background.

Case study

The symptoms of Patient I. (G.J., born on 30 Jan. 1973) began in 1993 with lower-limbs weakness and paraesthesia. The neurological condition showed the following symptoms: paraparesis, muscle strength 4/5, tendon reflexes in the lower limbs +3 and paraesthesia. The CT of the brain and the evoked potentials (EMG, ENG and SSEP) were negative. The condition of the patient improved spontaneously in 2 weeks, and accordingly no further examinations were performed. Two months after her second labour in March 1998, she was admitted because of limb weakness, nystagmus and ataxia. The neurological symptoms were grade I horizontal nystagmus, mild trunk ataxia, hemiparesis on the left side, muscle strength 4/5, tendon reflexes +4, and a positive Babinski reflex on both sides. EDSS score: 3 point (8). The MRI of the brain corresponded to demyelination (Fig. 1). The CSF findings pointed to local intrathecal synthesis (52%), IgG index: 1.31, cytology: 5/μl lymphocytes, total protein: 208 mg/l, albumin index: 3.9×10^{-3}. Oligoclonal bands (OCB) were detected with electrophoresis followed by IgG immunoblot (Fig. 2).

For quantitative analysis laser nephelometry and for qualitative analysis isoelectric focusing and immunoblotting were used. The degree of local intrathecal synthesis was calculated according to the Reiber formula (5). The somatosensory evoked potentials (n. medianus, n. ulnaris, n. tibialis and n. peroneus) revealed central myelin damage. The visual evoked potential showed extended latency on both sides. After the examination, the patient received megadose methylprednisolone (MP) therapy: 1 g MP intravenously for 3 days, followed by 1 mg/body weight/day MP orally for 11 days. Remission was achieved after the MP therapy. The patient was symptom-free

(EDSS score: 0 point). In July, after her second relapse, she received megadose MP therapy and achieved total remission.

Patient II. (G.O., born on 22 Jan. 1974) exhibited left-side optic neuritis at the initial examinations in February 1998. The visus at admission was 0.1 on the left side. The neurological condition was negative. The MRI findings on the head and optic nerve pointed to demyelination (Fig. 1). CSF findings pointed to local intrathecal synthesis (46%), IgG index: 1.25, cytology 5/µl lymphocytes, total protein: 335 mg/l, albumin index: 4.5 x 10^{-3}. OCBs were detected by electrophoresis (Fig. 2). Visual evoked potential showed extended latency on the left side P100: 154 msec, and P100: 98 msec on the right side. After the examination, the patient received megadose MP therapy; the visus on the left side improved to 1.

Patient III. (G.A., born on 8 Sept. 1976) was examined because of paraesthesia in all the extremities and vertigo. The only symptom in her neurological condition was the paraesthesia. EDSS score: 1 point. MRI of the brain corresponded to demyelination (Fig. 1.). CSF findings pointed to local intrathecal synthesis (70%), IgG index: 2.20, cytology: 4/µl lymphocytes, total protein: 315 mg/l, albumin index: 4.6 x 10^{-3}. OcBs were detected by electrophoresis (Fig. 2). Visual evoked potential showed extended latency on the left side P100: 142 msec, and P100: 138 msec on the right side. In consequence of her negative neurological condition despite the subjective complaints, she did not received MP therapy.

On the Poser (9) diagnostic criteria, all three sisters had definitive MS. Patient I. has relapsing-remitting form and Patient II. and III. have first attack MS patient. The neurological condition and the MRI examination of the parents were found to be

negative. We excluded previous neurological disease, and systematically taken medication during the last decades. All four living grandparents are healthy, as concerns the neurological findings.

Conclusion

Adoption studies suggest that the familial risk of MS susceptibility is influenced rather by genetic than by environmental factors (2, 10, 11). The influence of environmental factors is difficult to exclude, since the twins and siblings share the same environment. For the differentiation between the influence of genetic and environmental factor the population of adopted children and their parents have been used with success. A Canadian comprehensive study of recurrence has shown a lifetime risk of 0.2% for the entire population, which is increasing to 3% in other first degree relatives (relative risk 20) and 1% in second and third degree relatives (relative risk 5.5) (12). Comparison studies from the United Kingdom confirmed the highest recurrence rate for sisters (4.4%) and brothers (3.2%) compared with parents (2.8%) and offspring (1.8%). The reduction in risk changes from 2.8% in first-degree relatives to 1% and 0.9% in second and third degree relatives, compared with the background age adjusted risk in this population of 0.3% (13). Previously published data indicate that family risk range from 300-fold for monozygotic twins to 20-40-fold for biological first-degree relatives over the general population prevalence of 0.1% (10). The prevalence of familial MS in first-degree relatives is 5-10%, while that in monozygotic twins is 20-30% (6). These findings support the role of genetic factors in MS.

The role of environmental factors is greater in mono- than in dizygotic twins than in the first-degree relatives, which leads to confusion concerning the etiology (2). Adoption studies suggest that the familial environment play a role in the development of MS, while the frequency of the disease in non-biological relatives is equal to that in the normal population (10).

According to these findings, the familial occurrence of MS is genetically determined. We report on three affected sisters, whose parents and grandparents' medical history lack on any neurological or autoimmune disease. Taking into consideration that the familial environment does not necessarily lead to the disease, a genetic mutation of the sisters might be possible. Although the parents and grandparents are healthy, we can not exclude a common viral infection in their childhood nor other environmental factor, as a triggering factor of the disease. The crude risk for MS in the Northern European population is 1:600. If a sibling is affected, the risk increases to 1:40 (14). The possibility that in an unaffected family, in a medium-risk zone all three children would suffer form MS is less than 1:600. In the light of these data the case of these affected sisters might be interesting and their further follow-up, the encoding of the nature's over- or under-expression might lead to the better understanding of MS (14).

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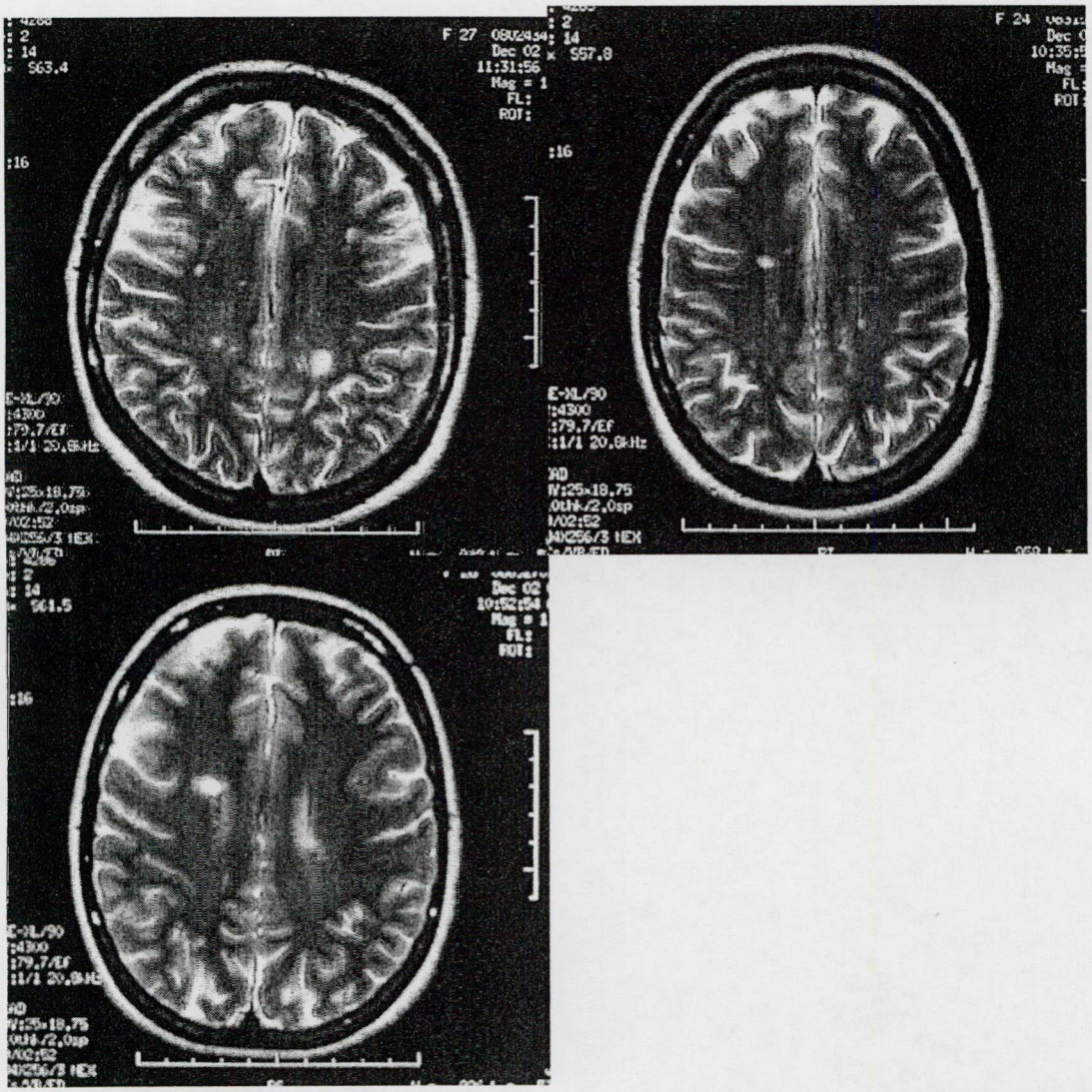
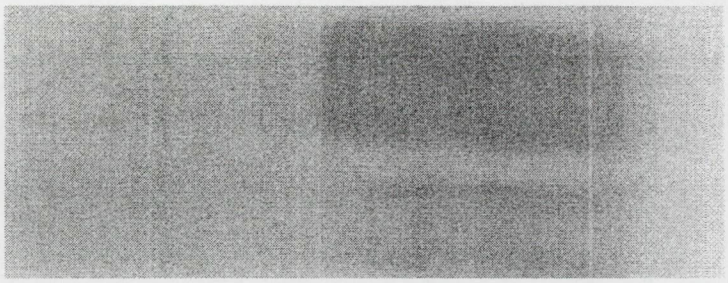


Fig. 1 MRI of the brain - corresponded to demyelination (Patient I, II, III)

Patient I.

CSF

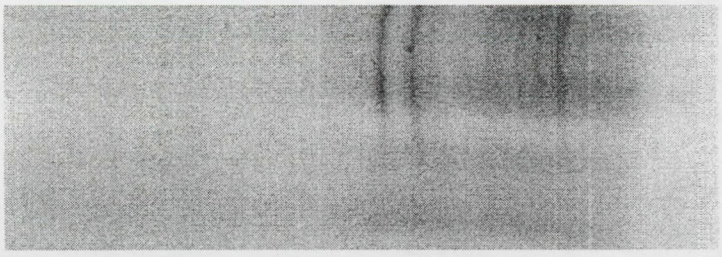
Serum



Patient II.

CSF

Serum



Patient III.

CSF

Serum

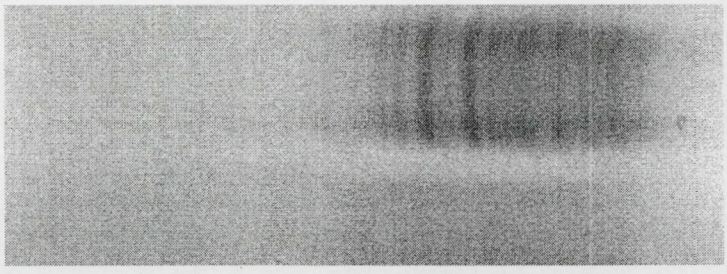


Fig. 2 Oligoclonal bands in cerebrospinal fluid detected by IgG immunoblot – order of appearance Patient II, Patient I, Patient III.